CLINICOPATHOLOGICAL FEATURES OF CUTANEOUS LYMPHOMAS AND T-CELL RECEPTOR GAMMA GENE REARRANGEMENT STUDIES IN EARLY STAGE MYCOSIS FUNGOIDES

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE RULES AND REGULATIONS FOR THE M.D. BRANCH XX (DERMATOLOGY, VENEREOLOGY AND LEPROSY) EXAMINATION OF THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY TO BE HELD IN APRIL, 2015

CERTIFICATE

This is to certify that the dissertation entitled “Clinicopathological features of cutaneous lymphomas and T-cell receptor gamma gene rearrangement studies in early stage mycosis fungoides” is the bonafide original work of Dr. Venkatraman.M.

This study was undertaken at the Christian Medical College and Hospital, Vellore from November 2012 to August 2014, under my direct guidance and supervision, in partial fulfilment of the requirement for the award of the MD degree in Dermatology, Venereology and Leprosy of the Tamil Nadu Dr. M.G.R. Medical University.

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Christian Medical College,

Vellore.

DECLARATION

I hereby declare that this M.D. dissertation entitled “Clinicopathological features of cutaneous lymphomas and T-cell receptor gamma gene rearrangement studies in early stage mycosis fungoides” is the bonafide work done by me under the guidance of Dr. Renu George, Professor, Department of Dermatology, Venereology and Leprosy, Christian Medical College, Vellore. This work has not been submitted to any other university in part or full.

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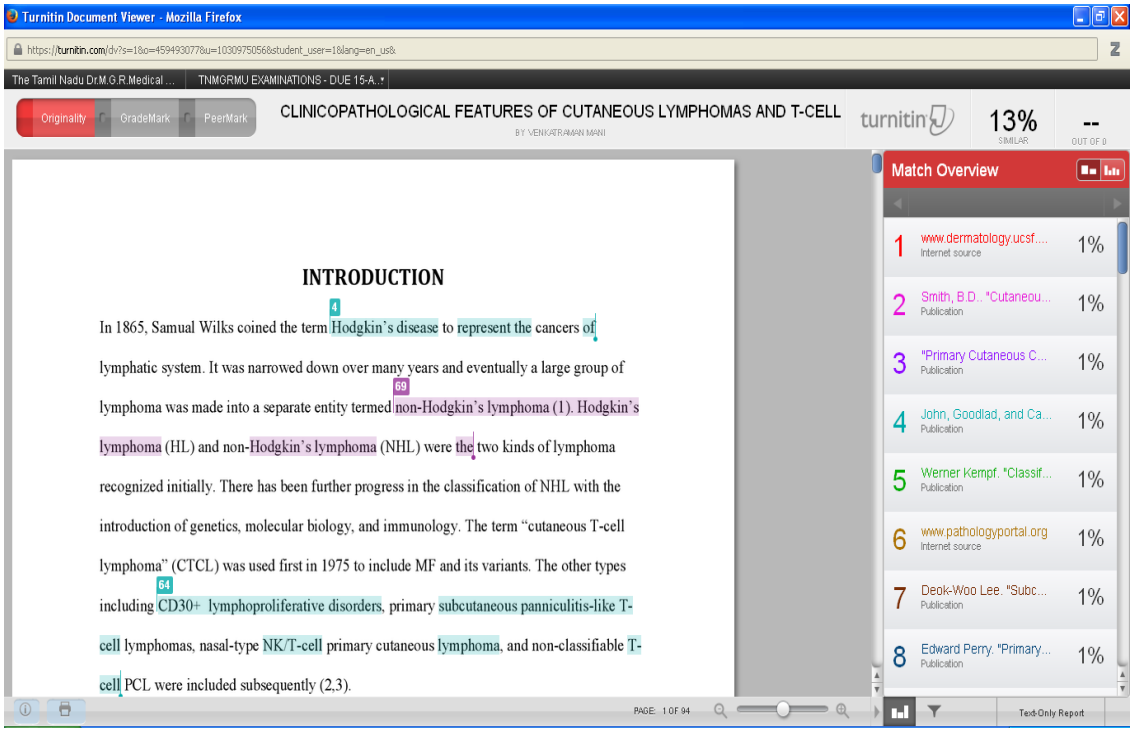
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INTRODUCTION
In 1865, Samual Wilks coined the term Hodgkin's disease to represent the cancers of lymphatic system. It was narrowed down over many years and eventually a large group of lymphoma was made into a separate entity termed non-Hodgkin's lymphoma (1). Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) were the two kinds of lymphoma recognized initially. There has been further progress in the classification of NHL with the introduction of genetics, molecular biology, and immunology. The term "cutaneous T-cell lymphoma" (CTCL) was used first in 1975 to include MF and its variants. The other types including CD30+ lymphoproliferative disorders, primary subcutaneous panniculitis-like T-cell lymphomas, nasal-type NK/T-cell primary cutaneous lymphoma, and non-classifiable T-cell PCL were included subsequently (2,3).
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Sub: **FLUID Research grant project NEW PROPOSAL:**
Clinicopathological features of cutaneous Lymphomas and T-Cell receptor Gamma Gene Rearrangement Studies in Early stage (StageI/IIA) Mycosis Fungoides
Dr. Venkatraman, PG Registrar, Dermatology, Dr. Renu George, Dermatology, Dr. Auro Viaswabandya, Hematology, Dr. Meera Thomas, Dr. Marie Therese, Pathology, Dr. Rekha Pai, Molecular Pathology

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Dear Dr. Venkatraman,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Clinicopathological features of cutaneous Lymphomas and T-Cell receptor Gamma Gene Rearrangement Studies in Early stage (StageI/IIA) Mycosis Fungoides" on November 6, 2012.

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Information Sheet and Informed Consent Form (English, Tamil, Hindi and Bengali)
3. Proforma
4. Cvs of Drs. Renu George, Auro Viaswabandya, Meera Thomas, Rekha Pai, Venkatraman.
5. A CD containing documents 1 - 4

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We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any serious adverse events occurring in the course of the project, any changes in the protocol and the patient information/informed consent. And on completion of the study you are expected to submit a copy of the final report.

A sum of Rs 40,000/- (Rupees Forty thousand only) will be granted for 12 months after receipt of the revised proposal. A subsequent installments of 40,000/- each will be released at the end of the first year following the receipt of the progress report (Total amount 80,000/-).

Yours sincerely

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TABLE OF CONTENTS

INTRODUCTION	**1**
AIMS AND OBJECTIVES	**3**
REVIEW OF LITERATURE	**4**
MATERIALS AND METHODS	**36**
RESULTS	**43**
DISCUSSION	**92**
CONCLUSIONS	**109**
LIMITATIONS	**111**
RECOMMENDATIONS	**111**
SUMMARY	**112**
BIBLIOGRAPHY	**116**
ANNEXURES	**124**
MASTER CHART	**169**

LIST OF TABLES

Table 1	**WHO-EORTC classification of cutaneous lymphomas**	**5**
Table 2	**Cutaneous lymphoma incidence pattern in various countries**	**7**
Table 3	**Gender distribution, mean age at diagnosis and 5 year survival rate of cutaneous lymphomas in various countries**	**9**
Table 4	**Frequency, age and gender distribution of cutaneous lymphomas in Indian studies**	**10**
Table 5	**Distinction between SPTL and PCGD-TCL**	**22**
Table 6	**Distinction between subtypes of PTCL, NOS based on immunohistochemistry**	**25**
Table 7	**Primary CTCL –Age at diagnosis and duration of disease prior to diagnosis**	**46**
Table 8	**Primary CTCL- Comparative clinical profile of each subtype of primary CTCL**	**47**
Table 9	**Relative frequency of salient histological features of MF**	**52**
Table 10	**Immunohistochemical features of MF**	**53**
Table 11	**Primary CTCL - Laboratory parameters, imaging studies, bone marrow, lymph node biopsy and organ biopsy**	**55**
Table 12	**TNM staging of MF**	**57**

Table 13	**Clonal detection rate in MF**	**58**
Table 14	**Relationship between histopathological diagnosis and clonality in early stage (I/IIA) MF**	**59**
Table 15	**Histological types of LyP**	**63**
Table 16	**Immunohistochemical markers pattern in CD30+ LPD**	**64**
Table 17	**TNM staging in LyP and cALCL**	**65**
Table 18	**Histological features in primary cALCL**	**67**
Table 19	**Histological and immunohistochemical features of SPTL**	**71**
Table 20	**'T' stage and TNM stage in SPTL patients**	**73**
Table 21	**Clinical, histological and immunohistochemical pattern in PTCL, NOS**	**76**
Table 22	**Demographic and clinical profile of secondary cutaneous lymphomas**	**82**
Table 23	**Histological features in various subtypes of secondary cutaneous lymphoma**	**83**
Table 24	**Immunohistochemistry pattern in various subtypes of secondary cutaneous lymphoma**	**84**
Table 25	**Frequency of subtypes of cutaneous lymphomas in different multicentre studies**	**93**

Table 26	**Comparison of demographic profile of cutaneous lymphomas with various studies done in India**	**95**
Table 27	**Comparison of gender distribution and mean age at diagnosis of cutaneous lymphomas across different countries**	**96**
Table 28	**Comparison of demographic profile of cutaneous lymphomas with various Institutions across the world**	**97**
Table 29	**Demographic profile of paediatric cutaneous lymphomas compared with studies across the world**	**98**
Table 30	**Clonality detection rate in early stage MF (I/IIA) among different studies using similar PCR protocol**	**101**
Table 31	**Comparison of clonality detection rate based on histology category among different studies**	**103**

LIST OF FIGURES

Figure 1	**Demographic profile of cutaneous lymphomas**	**43**
Figure 2	**Relative frequency of cutaneous lymphomas (primary and secondary)**	**44**
Figure 3	**Gender distribution in primary CTCL**	**45**
Figure 4	**Morphology of skin lesions in MF**	**49**
Figure 5	**Anatomical area of involvement in MF**	**50**
Figure 6	**Frequency of patients at different stages of MF**	**51**
Figure 7	**Relative frequency and gender distribution in primary CD30+ LPD**	**60**
Figure 8	**Various subtypes of secondary cutaneous lymphomas**	**80**

ABBREVIATIONS

ALK - Anaplastic lymphoma kinase

BPDCN - Blastic plasmacytoid dendritic cell neoplasm

cALCL - Cutaneous anaplastic large cell lymphoma

CBCL - Cutaneous B-cell lymphoma

CD30+ LPD - CD30 positive lymphoproliferative disorder

CD4+ PCSM-TCL - CD4 positive primary cutaneous small/medium sized pleomorphic T-cell lymphoma

CLA- Cutaneous lymphoid antigen

CTCL - Cutaneous T-cell lymphoma

DACLG - Dutch and Austrian Cutaneous lymphoma group

DDG - German Society of Dermatology

DLBCL - Diffuse large B-cell lymphoma

EBER - EBV encoded RNA

EMF - Erythrodermic mycosis fungoides

EORTC - European Organization for Research and Treatment of Cancer

ESR - Erythrocyte sedimentation rate

Extranodal NKTL - Extranodal Natural killer/T-cell lymphoma

HD - Heteroduplex

HIV - Human immunodeficiency virus

HL - Hodgkin's lymhoma

HPS - Haemophagocytic syndrome

HTLV-1 - Human T-cell lymphocytic virus-1

HVLL - Hydroa vacciniforme like T-cell lymphoma

IHC - Immunohistochemistry

ISCL - International Society for cutaneous lymphomas

IVLBCL - Intravascular large B-cell lymphoma

JSCS - Japanese skin cancer society

LDH - Lactate dehydrogenase

LMP-1 - Latent membrane protein -1

LyP- Lymphomatoid papulosis

MF- Mycosis fungoides

NHL- Non Hodgkin's lymphoma

PAGE - Polyacrylamide Gel Electrophoresis

PCDLBCLL - Primary cutaneous diffuse large B-cell lymphoma, leg type

PCFCL - Primary cutaneous follicle centre lymphoma

PCGD-TCL - Primary cutaneous gamma/delta T-cell lymphoma

PCMZL - Primary cutaneous marginal zone lymphoma

PCR - Polymerase chain reaction

PLEVA - Pityriasis lichenoides et varioliformis acuta

PTCL, NOS - Peripheral T-cell lymphoma, not otherwise specified

SEER - Surveillance, Epidemiology, and End Results

SPTL - Subcutaneous panniculitis like T-cell lytmphoma

SS - Sezary syndrome

TCR- T-cell receptor

TIA-1 - T-cell intracellular antigen-1

USA - United States of America

WHO - World Health organisation

ABSTRACT

Title of the abstract - Clinicopathological features of cutaneous lymphomas and T-cell receptor gamma gene rearrangement studies in early stage mycosis fungoides

Department	-	Department of Dermatology, Venereology and Leprosy
Name of the candidate	-	Venkatraman.M
Degree and subject	-	MD Dermatology, Venereology and Leprosy
Name of the guide	-	Dr Renu George

Abstract

Objective

To study the clinicopathological features of cutaneous lymphomas and the role of TCR gene rearrangement in the diagnosis ofpatch/plaque (Stage I/IIA) mycosis fungoides where the histological diagnosis of mycosis fungoides could not be excluded or the diagnosis was suggestive but not confirmatory.

Method

Ahospital based cross sectional study was conducted from November 2012 to August 2014 where in patients with a diagnosis of cutaneous lymphoma (CL)were included. Diagnosis was established by histology and immunohistochemistry and classified based on WHO-EORTC classification. TCR gamma gene rearrangement assay was done by PCR in patch/plaque (Stage I/IIA) mycosis fungoides (MF) where the histological diagnosis of mycosis fungoides could not be excluded or the diagnosis was suggestive but not

confirmatory. Routine laboratory tests; lymph node, bone marrow biopsies and imaging were done when indicated for staging based on the ISCL/EORTC proposal.

Results

There were 54 patients (M/F-1.25) with CL.This included primary cutaneous T-cell lymphomas (CTCL) (48), secondary CTCL (4), primary cutaneous B-cell lymphoma (CBCL) (1)and secondary CBCL (1). Among CTCL there were 27 cases of MF, LyP (lymphomatoid papulosis) (8), SPTL (subcutaneous panniculitis like T-cell lymphoma) (6), ALCL (Anaplastic large cell lymphoma) (3), PTCL, NOS (peripheral T-cell lymphoma, not otherwise specified) (3) and HVLL (hydroa vacciniforme like T-cell lymphoma) (1). Mean age at diagnosis of MF, CD30+ LPD, SPTL and PTCL, NOS were 41.1±18.8 years, 33.8±17.1 years, 33.6±17.3 years and 56.3±18.9 years respectively.Paediatric CL accounted for 16.7% of cases. The most common type of CTCL in the paediatric age group (≤20 years) was MF (n=4, 44.4%) followed by LyP (n=2, 22.2%), SPTL (n=2, 22.2%) and HVLL (n=1, 11.1%). Male preponderance was noted in all subtypes of cutaneous lymphoma except SPTL. Unusual clinical types of MF in our study included hypopigmented MF and dyshidrosiform MF. MF with palmoplantar involvement was seen in 14.8% patients. Among the unusual types of CTCL (other than MF) seen in our study were one case each of erythrodermic ALCL, LyP type D and HVLL. Clonality detection rate among early stage MF in our study was 61.5%. Statistical significance of clonal detection with histological grading and immunohistochemistry findings was not found in our study.

Conclusion

CTCL was the most common subtype as reported in earlier studies from Asian countries and the West. However the proportion of CBCL was lower than that reported from other countries. Female preponderance was noted in SPTL and rest of the subtypes showed a

male preponderance.MF was the commonest among CTCL comprising 56.25% of all CTCLs. Monoclonality detection in early stage MF was slightly lower than the detection rate in various studies across Europe using a similar protocol. Though the statistical significance of clonality detection with histopathological grading of MF could not be demonstrated it was evident that in patients with histopathological diagnosis suggestive of MF had increased clonal detection rate.Usefulness of TCR clonal rearrangement studies in early MF has to be further evaluated with large population studies.

Keywords

Cutaneous lymphoma, TCR gene rearrangement study, mycosis fungoides

INTRODUCTION

In 1865, Samual Wilks coined the term Hodgkin's disease to represent the cancers of lymphatic system. It was narrowed down over many years and eventually a large group of lymphoma was made into a separate entity termed non-Hodgkin's lymphoma (1). Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) were the two kinds of lymphoma recognized initially. There has been further progress in the classification of NHL with the introduction of genetics, molecular biology, and immunology. The term "cutaneous T-cell lymphoma" (CTCL) was used first in 1975 to include mycosis fungoides (MF) and its variants. The other types including CD30+ lymphoproliferative disorders, primary subcutaneous panniculitis-like T-cell lymphomas, nasal-type NK/T-cell primary cutaneous lymphoma, and non-classifiable T-cell PCL were included subsequently (2,3).

Mycosis fungoides was first described in 1806 in France by Alibert, who named it in 1814 as "Pian fongoide". The term "mycosis fungoides" was only adopted by Alibert in 1832 (4). It was Bazin, a student of Alibert who described its evolution according to the natural history of the disease and defined its stages as patch, plaque, tumoral and systemic dissemination in 1870. In 1938, Sezary and Bouvrain first described the Sezary syndrome (SS).

Considering all NHLs, B-cell NHLs are far more common than T-cell NHLs, (85% versus 15%), the reason being unknown. In cutaneous lymphomas the incidence is reversed. B-cell lymphomas are far less common than T-cell lymphomas (20-25% versus 75-80%) (5). This suggests that the development of cutaneous lymphomas is distinct from that of other lymphomas. Men are more commonly affected than women, with no identifiable risk factors or causes. NHLs are more common in adults and the

average age of diagnosis is in the early-to-mid 60s. Most of the cutaneous lymphomas are indolent (non -aggressive and slow growing). Most of the studies on cutaneous lymphomas are from the West, or countries like South Korea and Japan (6–9).

Well established cutaneous lymphomas are diagnosed by histopathological examination and immunohistochemical marker studies without much ambiguity. Molecular study (TCR gene rearrangement) plays an additional role in early lesions where the diagnosis of cutaneous lymphomas cannot be established confidently by the above modalities (10). Various studies around the world had demonstrated a wide range of clonality detection (40-90%) in CTCLs (11–15). Though the detection of clonality in early MF does not have any prognostic significance it has a significant role in diagnosis of early MF.

AIMS AND OBJECTIVES

Primary objective:

To study the clinicopathological features of primary and secondary cutaneous lymphomas.

Secondary objective:

To study the role of T-cell receptor gene rearrangement studies in the diagnosis of patch/plaque (Stage I/IIA) mycosis fungoides where in the histological diagnosis of mycosis fungoides could not be excluded or the diagnosis was suggestive but not confirmatory.

REVIEW OF LITERATURE

Cutaneous lymphomas (CL) are extranodal non-Hodgkin lymphoma of malignant, mature T and B lymphocytes that target the skin and have various distinct clinicopathological entities with varied natural histories, clinical presentations and treatment options (16). Owing to the rarity and heterogeneous presentation, it poses a great challenge in accurate diagnosis and subsequent treatment modalities. Cutaneous lymphomas may be primary or secondary. Of all the extranodal lymphomas, CL represent 19%, with mycosis fungoides being the commonest among them (6). Primary cutaneous lymphomas refer to those cutaneous T-cell lymphomas (CTCL) and cutaneous B-cell lymphomas (CBCL) without any extracutaneous disease at the time of diagnosis. Secondary CL represents infiltrates of a disseminated nodal or extranodal lymphoma into the skin. As aids to clinical management and prognostic evaluation, modern classifications tend to be based on clinicopathological entities (7). Based on clinicopathological findings, immunophenotyping is done for delineation and accurate diagnosis of variants of cutaneous lymphomas.

Cutaneous lymphomas with primary cutaneous manifestations were listed into 3 broad categories as follows based on World Health Organization–European Organization for the Research and Treatment of Cancer (WHO-EORTC) joint classification system [**Table 1**] which was updated in 2008 (7,17).

1) Cutaneous T-cell and NK cell lymphomas
2) Cutaneous B-cell lymphomas
3) Precursor hematologic neoplasm.

Table 1. **WHO-EORTC classification of cutaneous lymphomas**

WHO - EORTC CLASSIFICATION OF CUTANEOUS LYMPHOMAS (7)
Cutaneous T-cell and NK-cell lymphomas
Mycosis fungoides (MF)
Subtypes of MF
Pagetoid reticulosis
Granulomatous slack skin
Folliculotropic MF
Sezary syndrome
Adult T-cell leukemia/lymphoma
Primary cutaneous CD30+ lymphoproliferative disorders (CD30+ LPD)
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma* (SPTL)
Extranodal NK/T-cell lymphoma, nasal type (Extranodal NKTL)
Primary cutaneous peripheral T-cell lymphoma, unspecified (PTCL, NOS)
Primary cutaneous aggressive epidermotropic CD8+ T-cell lymphoma (provisional)
Cutaneous δ/γ T-cell lymphoma (provisional)
Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma(provisional)
Cutaneous B-cell lymphomas (CBCL)
Primary cutaneous marginal zone B-cell lymphoma (PCMZL)
Primary cutaneous immunocytoma
Primary cutaneous plasmacytoma
Follicular hyperplasia with monotypic plasma cells
Primary cutaneous follicle center lymphoma (PCFCL)
Growth patterns: follicular, follicular and diffuse, diffuse
Primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL)
Primary cutaneous diffuse large B-cell lymphoma, other
Intravascular large B-cell lymphoma (IVLBCL)
Precursor haematological neoplasm
CD4+ / CD56+ haematodermic neoplasm (formerly blastic NK cell lymphoma)

*By definition phenotype is T-cell receptor α/β chain positive

Epidemiology

The estimated annual incidence of cutaneous lymphoma (CL) is 1:100,000 according to the WHO (7). Most of the epidemiological studies on CL have been done in USA, Europe, Germany, Japan and Korea (6–9,18). A large population based study from US showed an increased incidence rate from 5.0/1000000 person-years during 1980-1982 to 12.7 in 2004-05 (6). Epidemiology of CL in Asia differs from the West with an increased incidence of extranodal NK/T-cell lymphoma, hydroa vacciniforme like lymphoma (HVLL), subcutaneous panniculitis like T-cell lymphoma (SPTL) and adult T-cell leukemia/lymphoma and low incidence rates of cutaneous B-cell lymphomas (19–21) [**Table 2**].

In Europe and USA, CTCLs constitute 75%–80% and CBCLs constitute 20%–25% of all cutaneous lymphomas (6,7). In Asia, the prevalence of CTCLs is higher than that reported from USA (6,8). A study from Japan showed a prevalence of mature T- and natural killer cell neoplasms of 85.7%, B-cell neoplasms were 12.9% and blastic plasmacytoid dendritic cell neoplasm was 1.4% (8). Another study from Korea showed a prevalence of primary and secondary cutaneous lymphomas of 74.2% and 17.9% respectively (9). Hospital based retrospective studies from India have shown the prevalence of CTCL to vary from 69% - 94% (22,23). Epidemiological data from multicentre studies are shown in **Table 2** (6–9,18).

Table 2. Cutaneous lymphomas incidence pattern in various countries

STUDY GROUP	DACLG	SEER	JSCS	KOREA	DDG
COUNTRY	NL+A (2005)	USA (2009)	JAPAN (2014)	KOREA (2014)	GER (2007)
Total no. of patients	1905	3884	1733	517	998
	Freq%	Freq%	Freq%	Freq%	Freq%
MATURE T-CELL AND NK-CELL NEOPLASMS	77	71.3	85.7	74.2	85
Mycosis fungoides (MF)	47	38.3	43.3	29.1	61
Sezary syndrome (SS)	3	0.8	1.9	0.3	2
Primary cutaneous CD30+ T-cell lymphoproliferative disorders	NA	10.2	12.0	NA	NA
Primary cutaneous anaplastic large-cell lymphoma (PCALCL)	8	NA	7.8	8.2	7
Lymphomatoid papulosis (LyP)	12	NA	3.8	8.5	7
Subcutaneous panniculitis-like T-cell lymphoma (SPTL)	1	0.6	2.0	8.2	NA
Peripheral T-cell lymphoma, Not otherwise specified (PTCL, NOS)	2	20.8	5.8	13.5	NA
Primary cutaneous CD4+ small/medium T-cell lymphoma	2	NA	1.4	1.3	5
Primary cutaneous δ/γ T-cell lymphoma	<1	NA	0.3	2.1	NA
Extranodal NK/T-cell lymphoma, nasal type (NKTL)	<1	0.3	2.3	7.1	NA
Adult T-cell leukemia/ lymphoma	NA	0.1	16.7	0.2	NA
MATURE B-CELL NEOPLASMS	23	28.5	12.9	17.9	15
Extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (PCMZL)	7	7.1	4.2	5.0	7

Primary cutaneous follicle center lymphoma (PCFCL)	11	8.5	2.1	1.1	4
Primary cutaneous diffuse large-cell lymphoma, leg type (PCLBCL, leg)	4	2.6	5.5	1.6	<2
Primary cutaneous diffuse large-cell lymphoma, other	NA	NA	NA	NA-	NA
IMMATURE HEMATOLOGICAL NEOPLASMS	NA	0.3	1.4	NA	NA
CD4+ / CD56+ haematodermic neoplasm (Blastic plasmacytoid dendritic cell neoplasm) (BPDCN)	NA	0.2	1.4	2.1	NA

Freq, Frequency; Figures in %; CL, Cutaneous lymphoma; DACLG, Dutch and Austrian Cutaneous Lymphoma Group; EORTC, European Organization for Research and Treatment of Cancer; DDG – German Society of Dermatology; JSCS – Japanese Skin Cancer Society; MF, mycosis fungoides; SS, Sezary syndrome; PCL, primary cutaneous lymphoma; PTL, peripheral T-cell lymphoma; SEER, Surveillance, Epidemiology, and End Results; WHO, World Health Organization; PCL, primary cutaneous lymphoma; MF, mycosis fungoides; SS, Sezary syndrome; NL+A, Netherlands and Austria; USA, United states of America; GER, Germany.

Gender distribution [Table 3]

Majority of studies have reported male preponderance in all subtypes of cutaneous lymphomas except SPTL (7–9). Equal gender distribution is noted in a study from Europe while rest of the studies had reported female preponderance in SPTL **[Table 3**]. A study from Japan has reported a female preponderance in NKTL and LyP (8). Male preponderance in cutaneous lymphomas has been reported in hospital based Indian studies also (22,23) [**Table 4**].

Table 3. Gender distribution, mean age at diagnosis and 5 year survival rate of cutaneous lymphomas in various countries

STUDY GROUP	DACLG (7)			SEER (6)			JSCS (8)			KOREA (9)
	M/F	AD	5 yr	M/F	AD	5 yr	M/F	AD	5 yr	M/F
MF	1.8	57.5	88	1.66	NA	90.9	1.41	62	NA	1.1
SS	NA	NA	24	2.11	NA	39.5	3.71	68	NA	NA
cALCL	2.5	NA	95	1.73	NA	73.1	1.62	67.5	NA	1.3
LyP	1.5	45	100	NA	NA	NA	0.83	53.5	NA	NA
SPTL	1	NA	82	NA	NA	NA	0.55	55	NA	0.5
PTL	NA	NA	16	1.82	NA	79.1	1.09	68	NA	1.1
NK/T	NA	NA	NA	NA	NA	NA	0.62	66	NA	1.5
PCMZL	NA	NA	99	1.28	NA	93.1	1.00	63	NA	1.9
PCFCL	NA	NA	95	1.84	NA	96.2	2.08	64	NA	NA
PCLBCL	NA	NA	55	1.70	NA	46.3	0.90	77	NA	1.9

NA – Not available; M/F – Male:Female ratio; AD – Age at diagnosis; 5yr – 5 year survival rate; MF, Mycosis fungoides; SS, Sezary syndrome; cALCL, Primary cutaneous anaplastic large cell lymphoma; LyP, Lymphomatoid papulosis; SPTL, subcutaneous panniculitis like T cell lymphoma; NKTL, Natural killer/T cell lymphoma; PTL, Peripheral T cell lymphoma unspecified; PCMZL – Primary cutaneous marginal zone lymphoma; PCFCL- Primary cutaneous follicular cell lymphoma; PCLBCL – Primary cutaneous diffuse large B cell lymphoma, leg type

Age distribution

A multicentre study from US has reported that the age incidence for MF, cutaneous CD30+ LPD and PTCL, NOS are similar among males and females at younger age (<30 years), but notably higher among males at older ages (6). Age at diagnosis of MF varies from 55-70 years in various studies while the same for CD30+ LPD varies from 40-55 years (6,8,9). Most of the cutaneous lymphomas are diagnosed

around 6th to 7th decade [**Table 3**]. Indian studies have shown a wide range of age distribution in MF (20-70 years) while the range of age distribution in CBCL was 35 - 70 years [**Table 4**]. SPTL occurs approximately 2 decades earlier than other types of primary cutaneous lymphoma and predominantly affects young adults (24).

Epidemiological and demographical data from Indian studies are tabulated as follows (22,23,25) **[Table 4]:**

Table 4. Frequency, age and gender distribution of cutaneous lymphomas in Indian studies

CL subtypes	George et al, 1999*			Doshi et al, 2011			Burad et al, 2014#		
	F %	Age range	M/F	F%	Age range	M/F	F %	Age range	M/F
MF/SS	39.4%	36-70	5.5:1	74.4%	20-39	3:2	36%	~	~
PCALCL	3.03%	NA	1:0	2.12%	NA	2:1	8.2%	~	~
LYP	NA	NA	NA	17%	41-60	2:1	8.2%	~	~
SPTL	9.09%	NA	NA	NA	NA	NA	24.5%	~	~
NKTL	NA	NA	NA	0.7%	NA	1:0	-NA	~	~
PTL	9.09%	NA	NA	NA	NA	NA	19.6%	~	~
AITL	3.03%	NA	NA	NA	NA	NA	NA	~	~
CBCL	21.1%	35-70	5:1	5.67%	41-60	1.6:1	NA	~	~

* Based on R.E.A.L classification; # Study included only peripheral T cell lymphomas; NA- Not available

Cutaneous lymphomas in children

Cutaneous lymphomas are uncommon in children. It accounts for around 6% of all malignant neoplasms in children. Two large studies on cutaneous lymphomas in children have been published from Europe and Korea (26,27). Proportion of paediatric

CTCLs in both studies were about 82% while that of CBCLs' proportion varied from 3% to 13% (26,27). MF was the commonest CTCL in Europe and LyP was the commonest in Korea (26,27). Median age at diagnosis of MF varies from 14–17 years while the same for LyP and SPTL varies from 8-14 years and 7-20 years respectively (26,27). The risk of developing lymphoid malignancies in children with LyP is similar to that of adults (28). Long term follow up is needed in children irrespective of its favourable prognosis. Primary cutaneous ALCL is rare in children however systemic ALCL can occur in approximately 15% of childhood NHLs. Secondary cutaneous involvement in systemic ALCL can occur in 18-25% children (29).

Cutaneous T-cell lymphomas

Mycosis fungoides

Mycosis fungoides (MF) is the most common CTCL subtype, with prevalence rate of 54% in US, 40-60% in Europe, 43% in Japan and 29% in Korea (6–9,18) [**Table 2**]. A recent retrospective study from India showed a prevalence of 34% of all CTCLs (25). Mycosis fungoides has a male predilection with M/F ratio between 1.6 and 2.1 (6–8). MF occurs more frequently in older individuals (>50 years) but recent studies had reported an increased incidence of MF in children and adolescents (16,30).

Risk factors

Male gender, black race and old age are the risk factors for the development of MF (6).

Anatomical distribution

Classical Alibert-Bazin type of MF presents with mild erythematous scaly annular or arcuate patches which classically involve sun-shielded areas ("bathing suit

distribution"). Though the early patches have predilection over unexposed areas, later they may become eczematous, hypopigmented or hyperpigmented. In a few patients erythroderma may arise *de novo* or from progression of patch/plaque MF. Patients who develop erythroderma and do not meet hematologic criteria for SS are considered as "erythrodermic MF" (EMF) (31).

Subtypes and variants

Subtypes and variants of MF include folliculotropic MF, pagetoid reticulosis and granulomatous slack skin. Other atypical variants including syringotropic, bullous/vesicular, hyperpigmented, hypopigmented, unilesional, palmoplantar, hyperkeratotic/verrucous, vegetating/papillomatous, ichthyosiform, pigmented purpura-like and pustular variants had been reported in literature. Few atypical variants including hypopigmented, pityriasis lichenoides-like, and ichthyosiform mycosis fungoides are more prevalent in Asians compared to the West (20). In particular hypopigmented MF which mimics pityriasis versicolor, pityriasis alba, vitiligo, leprosy and post inflammatory hypopigmentation had increasingly been reported in Asians (20).

Histopathology

Diagnosis of MF was supported by the following histopathologic findings which includes superficial lymphoid infiltrate, epidermotropism without spongiosis (intercellular edema of the epidermis), and atypical lymphoid infiltrate which is defined as cells with enlarged, hyperchromatic nuclei and irregular or cerebriform nuclear contours. The hallmark of MF and SS is epidermotropism, but in fact well-formed Pautrier microabscesses are seen in only a minority of patients (19%) and in most skin biopsies the diagnosis of early MF rests on other histological and clinical criteria (32).

The lymphoid infiltrate increases in density and invade the deeper reticular dermis, and subsequently nuclear atypia becomes more apparent as the disease progresses from early stage to advanced stages. Histological, immunological and molecular biology data as a criterion may be of interest for the dermatologist to differentiate early MF and other dermatoses. Presence of epidermal lymphocytes with extremely convoluted, medium–large (7-9μm) nuclei aids the correct diagnosis of MF which has 100% sensitivity and 92% specificity (33). In 1996, Muche et al was the first to demonstrate circulating neoplastic lymphoid cells in early stage MF, proving the molecular basis and systemic nature of the early MF (34). Guitart et al in 2001 proposed a criterion for diagnosis of MF (35). Major criteria included:

(1) Assessment of density of infiltrate at low power (mild perivascular, moderately dense perivascular or band like infiltrates and dense superficial infiltrate with widening of papillary dermis or involvement of deep reticular dermis)

(2) Epidermotropism at medium power (focal basal epidermotropism with or without Pautrier's microabscess, basal epidermotropism with 2 or more Pautrier's microabscess and extensive epidermotropism)

(3) Cellular atypia at high power (mild, moderate and uniform atypia).

Minor criteria included reticular fibroplasia of papillary dermis, intraepidermal atypical lymphocytes and lymphocytic infiltrate without inflammatory features. Based on the above criteria the diagnosis was classified into:

1. Mycosis fungoides
2. Atypical lymphocytic infiltrates suggestive of MF

3. Atypical lymphocytic infiltrates where the diagnosis of MF cannot be excluded and
4. Perivascular/interface dermatitis

Immunohistochemistry

Expression of CD4 which is a marker of mature helper T-cells is a hallmark finding of MF. Immunophenotypical markers which are commonly observed in MF includes CD2+, CD3+, CD4+, CD5+, CD45RO+, CLA+ (cutaneous lymphoid antigen), CD8- (cytotoxic T-cell marker) and CD30- (7). Low levels of CD7 expression (<10%) has a sensitivity of 40% and specificity of 80% to 100% which helps to distinguish early MF from benign mimics (41).

Course of the disease

Several studies had demonstrated that early MF patients with limited patches or plaques who have received skin-directed treatment have median survival similar to that of matched control populations (36–38). MF has an indolent clinical course with slow progression over years or sometimes decades, from patches to more infiltrated plaques and eventually to tumors. As MF progresses into advanced stages there is progressive growth of the neoplastic infiltrate into the dermis and subcutaneous fat. 25–33% of patient's progress to late-stage disease characterized by cutaneous tumours or erythroderma over months or years and nodal involvement may occur (36,39). Approximately one-third of patients present with advanced disease, which is associated with a poor prognosis (36,38). Around 6-28% of patients who had bone marrow involvement during the initial staging were associated with advanced skin and nodal disease (36,39).

Prognostic markers

Determination of prognosis is an essential step in the management of primary cutaneous lymphomas. The prognosis of patients with MF is dependent on few clinical markers such as male sex and increasing age, haematological markers such as LDH, histological features such as folliculotropism and genetic abnormalities apart from clinical staging, and in particular the type and extent of skin lesions and the presence of extracutaneous disease (7,39). Early stage disease has a favourable prognosis (5 year survival – 49-100%) while the advanced stage disease with tumours or erythroderma is associated with worse prognosis (5 year survival – 0-39%) (38–40). Variants like hypopigmented MF, MF with lymphomatoid papulosis, poikilodermatous MF and increased numbers of CD8+ T cells in histology of the skin are favourable prognostic factors and associated with reduced risk of disease progression (31,40).

Staging of MF

Staging guidelines was published in 2007 by ISCL/EORTC for MF/SS (Olsen et al) based on morphology of skin lesions, extent of involvement, nodal involvement, extracutaneous dissemination and blood involvement (41). (**Annexure IA & IB**)

'T' stage was divided into 4 stages (T1-T4) based on type of skin lesions and extent of involvement. T1 and T2 stages are further subdivided into 'a' and 'b' in respect to patch stage and plaque stage respectively. Patch stage disease has an improved survival than plaque stage MF (39,40). As the T stage of disease advances, prognosis of the disease proportionately becomes worse (40).

Clinical stage as defined by TNMB classification (IA-IVB) has prognostic implications and varied treatment approaches (39). Staging of the disease at presentation is a useful measure of tumour burden.

Mycosis fungoides in children

MF in children constitutes <5% of all MF cases (42). Male predominance is noted in children with MF similar to adults. In a study comprising 46 children with MF mean age at diagnosis was 10.3 years while the mean duration of symptoms prior to diagnosis was 24 months (30). Hypopigmented lesions are common in children than adults (30). Distinguishing MF from benign dermatoses particularly in hypopigmented lesions is a challenging task (30). Histological examination with immunohistochemical and molecular analysis may be helpful in such situation. The overall prognosis of MF in children is excellent with treatment (30).

Sezary syndrome (SS)

Sezary syndrome (SS), a triad of erythroderma, generalized lymphadenopathy, and the presence of neoplastic T cells (Sezary cells) in skin, lymph nodes, and peripheral blood has a poor prognosis (7).

Clinical features

SS usually occurs in a short time period de novo and progresses rapidly. Though it was considered separate from MF, rarely SS may follow classic MF (10). Patients with SS usually present with generalized exfoliative erythroderma with keratoderma and fissures on the palms and soles which may be associated with electrolyte imbalance, hypothermia, hair loss, and eyelid changes/ectropion (7).

Generalized seborrheic dermatitis, psoriasis, cutaneous drug reactions, infections and chronic photosensitivity reactions may mimic SS.

Diagnostic criteria

Only patients with SS will have susbstantial leukemic T-cell burden in the blood which helps to distinguish it from erythrodermic MF (43). The International Society for Cutaneous Lymphoma (ISCL) proposed these patients as ‘‘SS preceded by MF’’ to distinguish them from classic SS.

International Society for Cutaneous Lymphomas (ISCL) recommended criteria for diagnosis of SS include one or more of the following (43):

1) Absolute Sezary cell count of 1000 cells/cubic mm or more
2) Specific immunophenotypical abnormalities (CD4/CD8 ratio more than 10, loss of any or all of the T-cell antigens CD2, CD3, CD4, and CD5, or both)
3) Demonstration of a T-cell clone in the peripheral blood by molecular or cytogenetic methods.

Histopathology and immunohistochemistry

Classical histopathological features including epidermotropism, Pautrier microabscesses are variable and less prominent in SS. Traditional determination of Sezary cells quantification was largely replaced by flow cytometry analysis which identifies neoplastic T cells, which are characterized as CD4+ CD7- and/or CD4+ CD26- (44).

Prognosis

SS is an aggressive disease with a median survival of 2–4 years and an overall survival rate at 5 years of between 11 and 20% (7). Transformation into a large-cell lymphoma may also occur in the course of SS as in classical MF (43).

Primary cutaneous CD30+ lymphoproliferative disorders (CD30+ LPD)

The primary cutaneous CD30+ lymphoproliferative disorders (LPD) are the second most common form of CTCL contributing around 25% of all CTCLs'(45). Lymphomatoid papulosis (LyP) and cutaneous anaplastic large cell lymphoma (C-ALCL) form a spectrum of CD30+ lymphoproliferative disorders which is usually differentiated by clinical appearance, course and histological criteria. In India, the prevalence of cutaneous CD30+ lymphomas was reported to be around 16% of all CTCLs (25).

Lymphomatoid papulosis (LyP)

Clinical profile

LyP generally occurs in adults with male predominance but may occur in children as well. Median duration of skin lesions before first diagnosis was 18 months (range - 1 to 117 months) (45). Clinically, LyP is usually characterized by a chronic course of self-healing skin eruption of erythematous papules and nodules that may become haemorrhagic, necrotic, and/or ulcerative lesions each of which undergoes spontaneous regression after weeks or months (45). Conditions that resemble LyP are arthropod bites, pityriasis lichenoides *et* varioliformis acuta (PLEVA), prurigo nodularis, folliculitis and various other cutaneous lymphomas.

Histopathology

The three well recognized histological subtypes of LyP are (45)

i) Type A, characterized by a mixed infiltrate containing large atypical CD30+ cells intermingled with small lymphocytes, histiocytes, neutrophils and or eosinophils

ii) Type B, characterized by mycosis fungoides (MF) like histologic picture

iii) Type C, characterized by large CD30+ cells with relatively few admixed inflammatory cells which mimics anaplastic large T-cell lymphomas but has an indolent clinical course

Recently two new histological variants have been described. They include LyP type D in which the histology simulates an aggressive epidermotropic CD8 positive cytotoxic T-cell lymphoma but clinically follows an indolent course and type E, characterized by angioinvasive infiltrate of small to large atypical lymphoid cells which are CD30+, CD8+ and TIA-1+ which also follows an indolent course with an excellent prognosis (46,47).

Prognosis

It usually follows an indolent course and has an excellent prognosis (7). However, a cohort study has shown that 4% of patients develop systemic lymphoma and 2% of patients die of systemic disease over a median follow up period of 77 months (45). Twenty percent of patients LyP may be preceded by, associated with or followed by another type of malignant lymphoma, cutaneous ALCL or Hodgkin lymphoma (7).

Primary cutaneous anaplastic large cell lymphoma (primary cALCL)

Clinical picture

Primary cALCL manifests in majority of patients as a solitary or grouped, rapidly growing and ulcerating large tumors or thick plaques with associated extracutaneous spread in 10% (7). Rare presentation of erythrodermic cALCL has been reported (48). Median age at diagnosis has been reported to be 60 years in a study comprising 219 patients with CD30+ LPD (45). Male preponderance is noted in the same study. Nodules/tumors are the common morphological skin lesions in cALCL occurring in 88% of patients (45).

Histology

Histological picture of cALCL show a diffuse, non epidermotropic infiltrate with cohesive proliferations of large CD30+ lymphocytes. Neoplastic cells show anaplastic features in majority of patients and less commonly, they might have a pleomorphic or immunoblastic appearance (49). LyP, cALCL and secondary infiltrates of systemic ALCL as well as tumor stage of MF and transformation in SS show overlapping histological and phenotypical features and it exemplifies that differentiation can be achieved only by clinicopathological correlation and staging examinations (17). According to the latest WHO-EORTC classification, cALCL is classified by the expression of CD30 in more than 75% of large atypical cells (7). The atypical cells usually show an activated CD4+ T-helper cell phenotype with variable loss of T-cell markers and frequent expression of cytotoxic proteins. Borderline patients can also occur which include patients with the clinical presentation of a CD30+ cALCL but histologic features suggestive of LyP and conversely, patients with clinical picture of LyP that shows histologic features characteristic of a CD30+ cALCL (45).

The clinical presentation and the course of disease over time are used as decisive criteria for the definitive diagnosis and choice of treatment (49).

Prognosis

Primary cALCL has a favourable prognosis with 5-year survival rates between 76% and 96%. Spontaneous complete or partial regression of the tumor is reported in up to 44% of the patients (45,50).

Subcutaneous panniculitis like T - cell lymphoma (SPTL)

Subcutaneous panniculitis-like T-cell lymphoma (SPTL) which accounts for 1% of all CL's is defined based on new WHO/EORTC classification as a cytotoxic T-cell lymphoma showing an α/β+, CD3+, CD4-, CD8+ phenotype which is primarily limited to subcutaneous infiltrates with an indolent clinical course (7). Frequency of SPTL was reported to be around 8% among all peripheral T cell lymphomas in a study from an Indian tertiary referral centre (25). Only few other patient reports and a small patient series were reported from India till date (51–55).

In recent WHO-EORTC classification, SPTL and primary cutaneous γδ T cell lymphoma were distinguished as 2 groups in regards to distinct histology, phenotype and prognosis as tabulated below (7,56) [**Table 5**].

Clinical profile

The median age at diagnosis was 36 years (range 9-79 years) (56). Clinically SPTL occurs as nodular skin lesions or deeply seated plaques, which varies in sizes from 1 to 20 cm. Extremities are more commonly involved than trunk and face (56). The duration of skin lesions prior to the diagnosis ranged from 1 month to more than 10 years (56).

Table 5. Distinction between SPTL and PCGD-TCL

	SPTL	PCGD - TCL
IMMUNOHISTOCHEMISTRY	βF1+, TCR-γ1-, CD3+, CD4–, CD8+, CD56–	βF1-, TCR-γ1+, CD3+, CD4–, CD8–, CD56+
ARCHITECTURE	Subcutaneous	Subcutaneous and/or epidermal/dermal
HPS	Uncommon	Common
5 YR SURVIVAL	91% (without HPS) vs 46% (with HPS)	11%
TREATMENT	Systemic steroids	Systemic chemotherapy

HPS – Haemophagocytosis; TCR – T-cell receptor;

Histopathology

Typically all patients show a histological picture with predominant subcutaneous atypical lymphoid infiltrate, showing a typical adipotropism and characteristically involving the fat lobules resembling a lobular panniculitis (56,57). Septal involvement may be absent or appear as secondary changes. Mild to moderate extension of the atypical infiltrate into the reticular dermis and periappendageal infiltration was often observed (57).

Prognosis

Studies had shown CD56+, cytopenia with haemophagocytic syndrome, angioinvasion, skin ulcers, liver dysfunction, elevated LDH and EBER (EBV encoded RNA) oligonucleotides as unfavourable prognostic factors (56–58). When complicated by hemophagocytic syndrome (HPS), SPTL has an aggressive clinical course, and should therefore be treated with aggressive multiagent chemotherapy (56,58).

Extranodal NK/T – cell lymphoma (Extranodal NKTL)

Extranodal NK/T-cell lymphoma, nasal type, usually an EBV+ lymphoma of small, medium, or large cells with an NK-cell, or more rarely a cytotoxic T-cell, phenotype. After nasal cavity/nasopharynx, most common site involved is skin which may be a primary or secondary manifestation of the disease. Presence of extra nasal involvement is an important poor prognostic factor (59). Biopsy of the tumor usually shows a diffuse proliferation of lymphoid cells harboring a surface CD3–, cytoplasmic CD3e+, CD56+/– phenotype, and a cytotoxic profile (positive for TIA-1, perforin, or granzyme B). Demonstration of EBV-encoded RNA [EBER] or immunohistochemistry for latent membrane protein 1 (LMP-1) is mandatory for diagnosis (60).

Hydroa vacciniforme like T – cell lymphoma (HVLL)

Clinical profile

Hydroa vacciniforme (HV) is a rare condition of childhood, clinically characterized by necrotic papulovesicular eruption with scarring on sun-exposed areas which is considered to be associated with hypersensitivity to ultraviolet light. HVLL is an EBV+ lymphoproliferative disorder which has clinical similarities to HV was recognized by the 2008 WHO classification as HVLL (61). There are increased reports of this condition from Asia, Central and South America (62–64). Recurrent skin lesions may be indolent for even up to 15 years before it progresses to involve visceral organs which usually manifest as fever, asthenia, lymphadenopathy, and hepatosplenomegaly (65).

Histopathology

Histological findings are similar to chronic EBV infection which is characterized by NK-cell or T-cell infiltrates extending from epidermis to the subcutis, showing necrosis, angiocentricity, and angioinvasion. CD56 is positive only in the NK cell–derived lesions. Demonstration of EBER in situ hybridization is a definite criterion for diagnosis (62). Clonal T-cell rearrangement favours the diagnosis of HVLL (65).

Prognosis

Poor prognostic factors include presence of photosensitivity and symptoms indicating chronic active EBV infection, such as high viral load in the peripheral blood, clonality of EBV or T cells, deeper dermal infiltration and cellular atypia (66).

Peripheral T- cell lymphoma, not otherwise specified (PTCL, NOS) [Table 6]

All patients that do not belong to MF, SS, CD30+ LPD, SPTL or extranodal NK／T-cell lymphoma are assigned to the group of peripheral T-cell lymphoma, unspecified (PTL, NOS) according to the WHO／EORTC and WHO classifications (7,17). Male preponderance has been reported and median age at diagnosis is 68 years (range, 8-87 years). Median duration of skin lesions prior to diagnosis was 6 months in a study of 82 patients with PTCL, NOS (67). Most common morphological skin lesions were nodular or tumorous skin lesions. The skin lesions may occur as solitary skin lesions (26%) or localised (13%) or multifocal (61%) (67). Accounting for less than 10% of all primary cutaneous T-cell lymphomas, this was sub classified (provisional entities) (7) into:

1) Primary cutaneous aggressive epidermotropic CD8+ T-cell lymphoma
2) Primary cutaneous γ/δ T-cell lymphoma

3) Primary cutaneous CD4+ small/medium-sized/large pleomorphic T-cell lymphoma
4) Peripheral T-cell lymphoma, not otherwise specified

Table 6. Distinction between subtypes of PTCL, NOS based on immunohistochemistry

PTCL, NOS subtype	IMMUNOHISTOCHEMISTRY			
	CD4	CD8	TCR α/β	CM
Epidermotropic CD8+ T-cell lymphoma	+ or -	+	+	+
Cutaneous γ/δ T-cell lymphoma	-	- or +	-	+
Cutaneous α/β pleomorphic T-cell lymphoma	+ or -	-	+	+
Cutaneous medium/large pleomorphic T-cell lymphoma, not otherwise specified	+	+ or -	_	+

CM - cytotoxic molecule; TCR - T-cell receptor

Primary cutaneous γδ T cell lymphoma (PCGD-TCL) [Table 5]

Clinical picture

PCGD–TCL usually present with disseminated plaques and/or ulceronecrotic nodules or tumors with frequent involvement of mucosal and other extranodal sites. But lymph nodes or bone marrow involvement is uncommon.

Histopathology

The neoplastic infiltrates in PCGD-TCL are not confined only to the subcutaneous tissue, but generally involve the epidermis and/or dermis as well, which is in contrast to SPTL where the neoplastic infiltrates are confined to subcutaneous tissue (7). The neoplastic cells characteristically have a TCR γ/δ+, βF1–, CD3+, CD2+, CD5–, CD4–, CD8–, CD56+/– phenotype with strong expression of cytotoxic proteins. Commercially available TCR-γ antibodies have been extremely helpful in differentiating between PCGD-TCL and SPTL (60) [**Table 5**]

Prognosis

Hemophagocytic syndrome (HPS), characterized by an aggressive clinical course may occur particularly in patients with panniculitis-like tumors (60). PCGD-TCL has a poor prognosis and resistant to multiagent chemotherapy (60).

Primary Cutaneous CD8+ aggressive epidermotropic cytotoxic T-Cell Lymphoma

Clinical profile

Clinically these lymphomas are characterized by eruptive papules, nodules, and tumors with central ulceration and necrosis or superficial, hyperkeratotic patches and

plaques which tend to disseminate to mucosal and other extranodal sites (lung, testis, central nervous system, and oral mucosa), but lymph nodes are often spared (60).

Histopathology

Ranging from a lichenoid pattern with marked, pagetoid epidermotropism in early patch-like lesions to diffuse dermal infiltrates in nodular and tumorous lesions in which epidermotropism and folliculotropism are marked, histological picture is variable (67). Tumor cells may be small medium or medium-large with pleomorphic or blastic nuclei. They have a βF1+, CD3+, CD8+, granzyme B+, perforin+, TIA-1+, CD45RA+, CD45RO–, CD2–, CD4–, CD5–, CD7–/+ T-cell phenotype (67).

Prognosis

It expresses an aggressive clinical behaviour and poor prognosis. Angiocentricity and angioinvasion may be present (67).

Cutaneous small/medium sized pleomorphic T-cell lymphoma, not otherwise specified (CD4+ PCSM-TCL)

Clinical picture

CD4+ PCSM-TCL, characterized by predominance of small to medium-sized CD4+ pleomorphic T cells occurs without prior or concurrent patches and plaques typical of MF (7). It usually present with a solitary plaque or tumor localized to the head or neck. The prognosis of such patients are excellent (60).

Histopathology

Histologically, these lymphomas show nodular to diffuse infiltrates, which often extend into the subcutaneous fat. IHC shows a predominance of CD3+, CD4+, CD8–,

CD30– small to medium pleomorphic T cells and fewer than 30% large CD4+ pleomorphic T cells (7). In most of the patients small reactive CD8+ T-cells are admixed with CD20+ B-cells, including some centroblasts and histiocytes and in some patients with multinucleated giant cells and/or granulomatous changes. The clinical presentation, architecture, and cellular composition of PCSM-TCL are similar to pseudo-T-cell lymphomas (60).

Cutaneous B-cell lymphomas (CBCL)

Cutaneous B cell lymphomas always remain a great challenge for the clinicians and pathologists in terms of definitive diagnosis and treatment. The different types of cutaneous B cell lymphomas include primary cutaneous marginal zone B cell lymphoma (PCMZL), primary cutaneous follicle centre lymphoma (PCFCL), primary cutaneous diffuse large B cell lymphoma leg type (PCLBCL–LT), primary cutaneous diffuse large B-cell lymphoma, other and intravascular large B-cell lymphoma (7). Primary cutaneous follicle centre lymphomas are the most common among CBCLs' (68). Studies have shown that B-cell lymphomas are rare in Asia compared to the West (6,7,69). Data about cutaneous B cell lymphomas reported from India are very sparse except few case reports (68,70–72). Three essential steps in diagnosis of cutaneous B-cell lymphomas include the determination of infiltrates (benign or malignant), identification of cell lineage and the final step is classification of B- cell lymphoma (73). Evidence of absence of extracutaneous disease for 6 months after diagnosis is required to classify as primary cutaneous B cell lymphomas (7).

Primary cutaneous follicle center lymphoma (PCFCL)

Clinical picture

PCFCL usually presents as solitary or grouped papules, plaques, or nodules with scalp, forehead, neck, and trunk being the more common sites of involvement and rarely occurs on lower extremities. Median age at diagnosis is 60 years and men are affected 1.05 times more than women (74). Incidence of PCFCL is around 9-11% of all cutaneous lymphomas (6,7). Benign inflammatory conditions including rosacea, folliculitis, acne, lupus miliaris, epidermal inclusion cysts, arthropod bites, benign cutaneous neoplasms and malignant neoplasms including basal cell carcinoma, Merkel cell carcinoma, and other non B-cell cutaneous lymphomas or folliculotropic MF can mimic PCFCL.

Histopathology

Histological patterns include follicular, nodular, diffuse, and mixed patterns which do not differ much in terms of prognosis (73,74). Histopathological examination of PCFCL usually shows dermal and subcutaneous proliferation of cleaved follicle center cells (centrocytes) admixed in variable proportions with large transformed cells (centroblasts). Immunophenotyping of follicle center cells generally express CD20+, CD79a+, Bcl-6+, Bcl-2- and variable expression of CD43 and CD10 (73).

Prognosis

Five-year survival rate of PCFCL is up to 95% and 5-10% of patients can disseminate to extracutaneous sites (7,73). Leg lesions carry a poor prognosis (68).

Primary cutaneous marginal zone lymphoma (PCMZL)

(Extranodal MZL of MALT lymphoma – WHO 2008 classification)

Clinical picture

PCMZL's are indolent lymphomas which commonly present as plaques and nodules with cutaneous recurrences but rare extracutaneous dissemination. PCMZL contributes 2-7% of primary CL and can present as solitary or localized lesions which affect multiple anatomical sites simultaneously or rarely be generalized (75). Most common anatomical sites to be involved are extremities and trunk rather than head and neck. Median age at diagnosis is 55 years with an excellent prognosis and a 5-year survival of 90-100% (7).

Histopathology

Prominent histological features include dermal and subcutaneous proliferation of marginal zone B-cells, lymphoplasmacytoid mononuclear cells, and plasma cells admixed with a minor number of centroblast- or immunoblast-like cells and many reactive T-cells. In patients with abundant plasmacytoid cells, PAS positive, immunoglobulin containing intranuclear Dutcher bodies and intracytoplasmic Russell bodies may be present (75). IHC usually shows CD20+, CD43+, CD10- , CD5-, bcl-2+, and bcl-6-. Multiple myeloma-1/interferon regulatory factor-4 (MUM1/IRF4) and CD138 positivity may be noted reflecting the plasma cell component of the infiltrate (75).

Primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL-LT)

Clinical picture

PCLBCL-LT constitutes about 10-20% of all PCBCL's and presents as multiple red or bluish red nodules or tumors and infrequently as solitary lesions. It occurs more common in women and elderly, with a median age of onset of 76 years (73). Presence of multiple lesions at diagnosis is a poor prognostic indicator. 5 year disease specific survival is about 50% (7,76).

Histopathology

Histology of PCLBCL-LT shows a diffuse dermal infiltrate of centroblasts and immunoblasts in monotonous or confluent sheets. IHC usually displays CD20+, CD79a+, CD10-, Bcl-2+ and Bcl-6+/-. Expression of Bcl-2, MUM1/IRF4, and FOX-P1 are strong in contrast to PCFCL (75).

The term "**PCLBCL, other**," refers to rare patients of large B-cell lymphomas that lack the typical clinical, histological and immunophenotypical features of PCLBCL-LT (17). Unlike their nodal counterparts, they appear to have an excellent prognosis (76).

Intravascular large B- cell lymphoma, characterized by an accumulation of large neoplastic B cells within blood vessels carries a poor prognosis. Central nervous system, lungs and skin are preferentially affected. Pure cutaneous involvement has relatively a better prognosis (77).

B-cell lymphomas associated with EBV infection are diffuse large B-cell lymphomas, plasmablastic lymphoma and plasmacytic marginal zone lymphoma which are usually related to immunosuppression (78).

Precursor hematologic neoplasm

CD4+/CD56+hematodermic neoplasm (formerly blastic NK-cell lymphoma)

Commonly presents as solitary or multiple nodules or tumours with or without concurrent extracutaneous localizations which exhibits an aggressive course with a poor prognosis. Median survival is around 14 months. Bone marrow, peripheral blood, lymph nodes, and extranodal sites can be rapidly involved in patients presenting with cutaneous lesions (7,79,80).

Secondary cutaneous lymphomas

B- Cell lymphomas

In contrast to primary cutaneous lymphoma, B-cell predominance (65.8%) is seen in secondary cutaneous lymphomas. It represents 25% of all cutaneous lymphomas (69). In one study, it contributes about 50% of cutaneous lymphomas other than MF. Clear distinction between primary and secondary lymphomas can be difficult because of incomplete clinical evaluation in most of the situations. Based on published literature most common secondary cutaneous B- cell lymphoma is diffuse large B-cell lymphoma which accounts for 26.8%, next being follicular lymphoma (21.4%) in which combined expression of bcl-2, bcl-6 and CD10 appears to a much strong predictor of secondary disease (68). Though primary and secondary diffuse large B cell lymphomas commonly involve the head and neck regions or the extremities, trunk involvement may be more characteristic of secondary disease (81). Clinical behaviour and gene expression profiling are much helpful in distinction between primary and secondary types (73). Expression of CD10 or t (14;18) warrants a systemic evaluation

(73). In patient of marginal zone lymphomas, secondary disease appears to be more commonly associated with multifocal skin disease when compared to primary marginal zone lymphomas (74).

T and NK cell lymphomas

19-55% of PTCL, NOS patients can have secondary cutaneous involvement at the time of diagnosis. Most common among secondary T and NK cell lymphomas is PTCL, NOS which is clinically aggressive than primary cutaneous PTCL. Study from India had reported a frequency of 34% patients of PTCL, NOS with cutaneous involvement (25).

Secondary CD30+ lymphoproliferative disorders accounts for 5% of all CD30+ LPD with median age of 55 years at diagnosis and bimodal age distribution (45). Anaplastic large cell lymphoma which accounts for around 9.1% has worse prognosis compared to its primary counterpart which is relatively indolent (45). In contrast to systemic anaplastic large-cell lymphoma (ALCL), primary CD30+ cALCL rarely carries the t(2;5) translocation and is usually ALCL kinase protein (ALK) negative (45).

Around 8 to 20% of NK/T cell lymphomas of the nasal type can have secondary cutaneous involvement (82). As in any other sites, infiltrate is composed of medium to large irregular cells along with angioinvasion and necrosis. Cells usually express CD2+, cytoplasmic CD3+, CD56+ and EBV positive. Skin lesions commonly occur in angioimmunoblastic T cell lymphoma (AITL), maculopapular eruptions resembling viral exanthem being the commonest presentation (82–84). Histologically the skin lesions may have sparse infiltrate with fairly nonspecific perivascular lymphocytes and eosinophils and associated capillary hyperplasia, with or without obvious abnormalities

of the lymphocytes. Most of the patients have enlarged, atypical lymphoid cells with loss of aberrant T- cell antigens.

Among HTLV-1 associated lymphomas most common is adult T-cell leukemia/lymphoma with life time risk being 1-4% and latent period for development of disease is estimated to be 30-50% (85). Histopathologically poor prognosis is noted in patients with nodules diffuse infiltration of medium sized to large lymphoma cells.

TCR gamma gene rearrangement

Well established cutaneous lymphomas are diagnosed by histopathological examination and immunohistochemical marker studies without much ambiguity. Molecular study (TCR gene rearrangement) plays an additional role in early lesions where the diagnosis of cutaneous lymphomas cannot be established confidently by above modalities (10). Various studies around the world had demonstrated a wide range of clonality detection (40-90%) in CTCLs' (11–13,86). But the difference is attributed to the usage of different PCR protocols for the detection of clonal cells. One study from Italy which had used multiplex PCR /HD analysis of PAGE on fresh frozen sections had demonstrated clonality in 83.5% of CTCLs in a large series of patients (12). Clonality detection rate proportionately increased with 'T' score of the disease (12). Ponti et al demonstrated a clonality percentage of 83.5% in all CTCLs and 73.75% in T1/T2 stages of MF (87). Tok et al had demonstrated clonal TCR gamma gene rearrangements in 73% of the specimen's non diagnostic for CTCL, 71% of those suggestive of CTCL, and 74% of those diagnostic of CTCL (87). Another study from China had demonstrated clonal detection of 80% and 100% in cases of suspected MF and typical MF respectively (88).

Though the detection of clonality in early MF does not have any prognostic significance, it has significant role in diagnosis of early MF (89). False negativity in detection of clonality can occur due to low density of infiltrated tumour cells in biopsy specimens of early MF, partial or incomplete rearrangement of the TCR, somatic hypermutation or TCR region translocation (86,90). There are no studies on clonality (TCR gamma gene rearrangement) of early stage mycosis fungoides from India till date.

MATERIALS AND METHODS

Setting

The study was conducted at the Department of Dermatology, Venereology and Leprosy, Christian Medical College and Hospital (CMCH). Patients with cutaneous lymphoma who presented to Dermatology OPD and patients referred mainly from the adult and paediatric Haematoncology and Medicine departments were included in the study.

Study design

A hospital based cross-sectional study was conducted where in patients fulfilling the inclusion criteria were recruited into the study.

Study duration

The study was conducted from November 2012 to August 2014 (22 months)

Inclusion criteria

Any patient with cutaneous lymphoma diagnosed by histopathological examination and immunohistochemistry as per standard criteria.

Exclusion criteria

Patients who were not willing to be included in the study.

METHODS

Patients who fulfilled the inclusion criteria were included in the study. All patients who were diagnosed to have cutaneous lymphomas by histopathology and immunophenotyping seen during the study period were included in the study. All the subjects and the parents/legal guardian of the subjects were informed about the purpose of the study (**Annexure IV-VI**) as well as informed consent was obtained (**Annexure VII-IX**)

All the patients were examined by the principal investigator and the details obtained from the patient or parent/legal guardian were entered in a proforma (**Annexure XIIA**). The details were collected and subsequently entered in an electronic database. Epidata (version 3.1), a data management software was used for the data entry and evaluation.

Demographic details

Socio-demographic data including age at recruitment into the study, gender, place of stay, duration of symptoms and age of onset were recorded. Age of onset was defined as the age at which the patient first noticed the skin lesions.

History pertaining to the disease

1. Presenting cutaneous complaints (including skin lesions, pruritus and other complaints) and duration of the same were recorded.
2. Systemic complaints (including history of fever, night sweats and weight loss) associated with the primary complaints were recorded.
3. Past treatment of the patient (if any) including systemic and topical medications were recorded.

Clinical Examination

Each patient was examined by the principal investigator and the findings were confirmed by the guide. Morphology of skin lesions, area of involvement and the extent of involvement were recorded. The diagnosis of the lesions was based on clinical features and confirmed by skin biopsy in all patients. The systemic examination including presence of pallor, lymph node assessment, organomegaly and ascites (if present) were recorded.

Investigations

Histopathological examination

Skin Biopsy

Skin biopsy from the most advanced lesion was done and in those with varied morphology the most common representative lesion was chosen for biopsy. Wedge and elliptical biopsies were done from ulcers and subcutaneous nodules and plaques.

Haematoxylin and eosin stain

The biopsy sample obtained was fixed, processed in an automated processor for thirteen hours and embedded in paraffin wax, and each tissue specimen was serially sliced into sections of 4 μm for routine haematoxylin and eosin staining.In each biopsy the following parameters were analysed;

(1) Epidermal changes (hyperplasia, ulceration, etc.)

(2) Epidermotropism

(3) Pattern and thickness of the lymphoid infiltrate

(4) Presence of Pautrier's microabscess

(5) Papillary dermis thickening

(6) Presence of spongiosis

(7) Presence of an inflammatory component (plasma cells, histiocytes, eosinophils, etc)

(8) Presence of mitoses.

Percentage of large T-lymphocytes was also analysed (>25% was considered as significant).

Diagnosis of MF

The diagnosis of mycosis fungoides was based on the histologic criteria proposed by Guitart et al (35) (**Annexure II**). Major criteria included assessment of density of infiltrate at low power, epidermotropism at medium power and cellular atypia at high power. Minor criteria included reticular fibroplasia of papillary dermis, intraepidermal atypical lymphocytes and lymphocytic infiltrate without inflammatory features. Based on the above criteria the diagnosis was classified into:

1. Mycosis fungoides
2. Atypical lymphocytic infiltrates suggestive of MF
3. Atypical lymphocytic infiltrates where diagnosis of MF cannot be excluded and
4. Perivascular/interface dermatitis.

Diagnosis of cutaneous lymphomas other than MF/SS

Cutaneous lymphomas other than MF/SS diagnosed by the distinct histopathological features and immunohistochemistry markers were classified based on WHO-EORTC classification (updated in 2008) (7,17).

Immunohistochemistry studies

Immunohistochemistry was done manually using the Envision technique developed with diaminobenzidine for all patients on formalin fixed paraffin embedded tissue sections. A primary panel of one pan T-cell marker (CD3), one pan B-cell marker (CD20) and proliferation marker (MIB1) was used. In CD3 positive patients other markers were done for sub typing depending on the morphology which included pan T-cell antigens CD5 and CD7 and subset markers CD4 (helper T cell antigen) and CD8 (cytotoxic T cell antigen). NK cell antigen CD56 and EBV antigen LMP-1 were done for patients with clinical and morphological findings suggestive of NK/T cell lymphoma. In patients with morphology and clinical background of CD30+LPD, activation marker CD30 and ALK epithelial membrane antigen (EMA) were done. The details of the antibody panels used including their clones, source, dilution and pre-treatment have been shown in annexure X (A). Procedure of immunohistochemistry has been shown in annexure X (B).

TCR gene rearrangement studies (Annexure XI)

TCR gamma gene rearrangement study was done in patch/plaque (Stage I/IIA) mycosis fungoides where in the histological diagnosis of mycosis fungoides could not be excluded or the diagnosis was suggestive but not confirmatory. Additionally the test was done in other stages of MF and few subtypes of cutaneous lymphoma if requested by the referring physician of the patient.

DNA was extracted using the Qiagen DNA Mini kit (Qiagen, Hilden, Germany). Three- four 5µ thick paraffin embedded sections were used. The 260/280 ratio was determined using the nanodrop (Nanodrop technologies, USA). Fifty ng of DNA was amplified to determine the quality of DNA using the ‘amplification control’

provided along with the T cell receptor gamma chain gene rearrangement assay (*In Vivo* Scribe, USA). Further, mixes 1 and 2 were amplified as per manufacturer's instructions. The products were detected on a 2% agarose gel before processing them for fragment analysis. Fragment analysis was performed using 1µl of the PCR product that was denatured with formamide and finally detected using the 3130 genetic analyser (Applied Biosystems, USA).

Other biopsies

Bone marrow biopsies, lymph node biopsies and extracutaneous organ biopsies were done in patients whenever indicated as per ISCL/EORTC staging protocol (**Annexure IA & IIIB**).

Laboratory tests

It included a complete haemogram, liver function test, creatinine, urinary analysis and serum level of lactate dehydrogenase. Blood borne virus screening (optional) included the screening for hepatitis-B, hepatitis-C and HIV.

Imaging studies

Imaging studies included chest X-ray, ultrasonography of abdomen and pelvis, computed tomography, magnetic resonance imaging and positron emission tomography. Imaging studies were done whenever indicated as per staging protocol.

Staging

All the patients were classified based on WHO-EORTC classification of cutaneous lymphomas (2005). Complete staging was done wherever possible based on the International Society for Cutaneous Lymphomas (ISCL)/European Organisation of

Research and Treatment of Cancer (EORTC) revised clinical staging and classification of mycosis fungoides and Sezary syndrome (41) (**Annexure IA & B**). Patients other than MF/SS were staged based on ISCL/EORTC proposal on TNM classification of cutaneous lymphoma other than MF/SS (91). (**Annexure IIIA**)

Sample size

As it is a descriptive study all consecutive consenting patients with a diagnosis of cutaneous lymphomas during the study period between November 2012 and August 2014 attending Dermatology unit I out-patient department and referred from other departments were recruited in the study. TCR gene rearrangement was done on patients with early stage MF (I/IIA) in whom the diagnosis of MF was not confirmatory.

Statistical analysis

All numerical variables were described using mean and standard deviation. Categorical variables were summarized using frequencies and percentages. *P* value was calculated using Fisher's exact test and Kruskal-Wallis equality-of-populations rank test wherever applicable.

Study approval

The study was approved by Institutional Review Board (Research and Ethics committee) [IRB no 8050].

RESULTS

Demographic profile

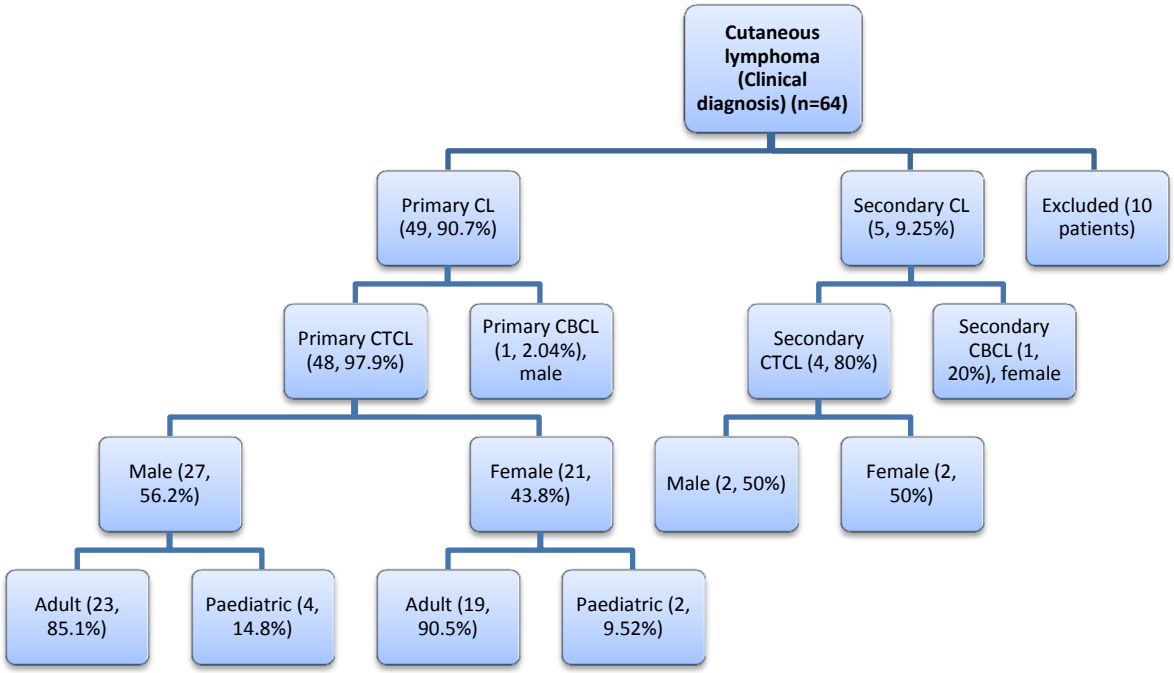


Figure 1.Demographic profile of cutaneous lymphomas

Fifty-four patients with diagnosis of cutaneous lymphomas were included in the study between November 2012 and August 2014 (22 months) [**Figure 1**].

Geographical location

The patients were from Tamilnadu (n=16, 29.6%), West Bengal (n=9, 16.6%), Kerala (n=6, 11.1%). Remaining patients were from Andhra Pradesh (n=5, 9.25%), Jharkhand (n=5, 9.25%), Chhattisgarh (n=4, 7.4%), North Eastern states (n=3, 5.55%), other states (n=5, 9.25%) and the neighbouring country Bangladesh (n=1, 1.85%).

Age and gender distribution

The patients included in the study ranged from 2-77 years (median, 40.7 years). Mean age at diagnosis of cutaneous lymphomas was 39.9±18.3 years (range 2-77

years).There were 30 males and 24 females (M/F ratio, 1.25). M/F ratio of primary CL and secondary CL were 1.33 and 0.5 respectively [**Figure 1**]. Six (11.1%) patients belonged to paediatric age group (age≤16 years) (M/F 2:1).

Subtypes of cutaneous lymphomas

There were 49 patients (90.7%) of primary CL and 5 patients (9.26%) of secondary CL. The primary CL included 48 patients (88.9%) of primary CTCL and one patient (1.85%) of primary CBCL. The secondary CL included 4 patients (7.4%) of secondary cutaneous T-cell lymphoma and one patient (1.85%) of secondary CBCL [**Figure 2**].

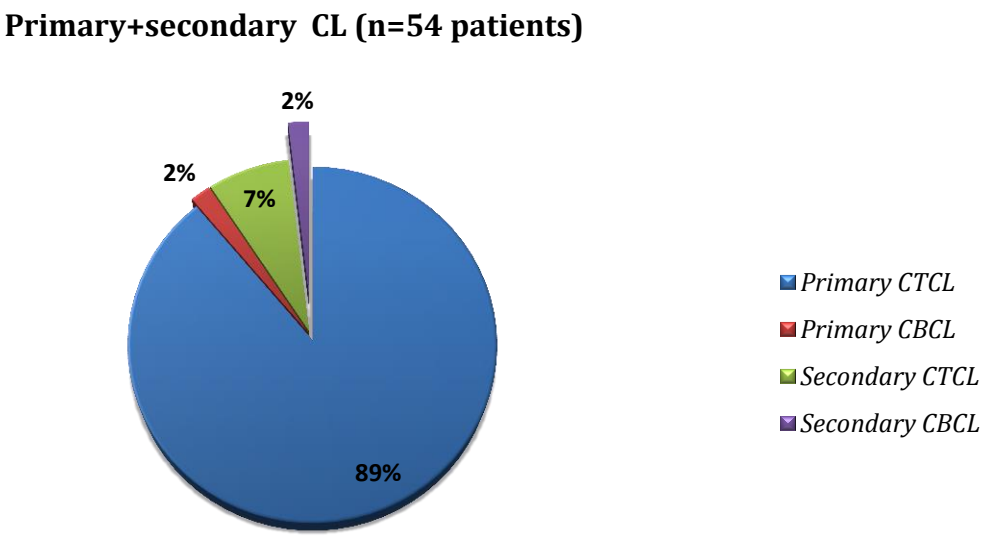


Figure 2.Relative frequency of cutaneous lymphomas (primary and secondary)

Primary cutaneous T-cell lymphomas

Forty-eight patients had primary CTCL, of which MF (n=27, 56.25%) was the most common type. Other subtypes in decreasing order were CD30+ lymphoproliferative disorders (CD30+ LPD) (n=11, 22.9%), SPTL (n=6, 12.5%), peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS) (n=3, 6.25%) and hydroa vacciniforme like T-cell lymphoma (HVLL) (n=1, 2.08%). There were 27 males and 21 females (M/F ratio - 1.28). Male predominance was noted in all subtypes of primary CTCLs except SPTL [**Figure 3**].

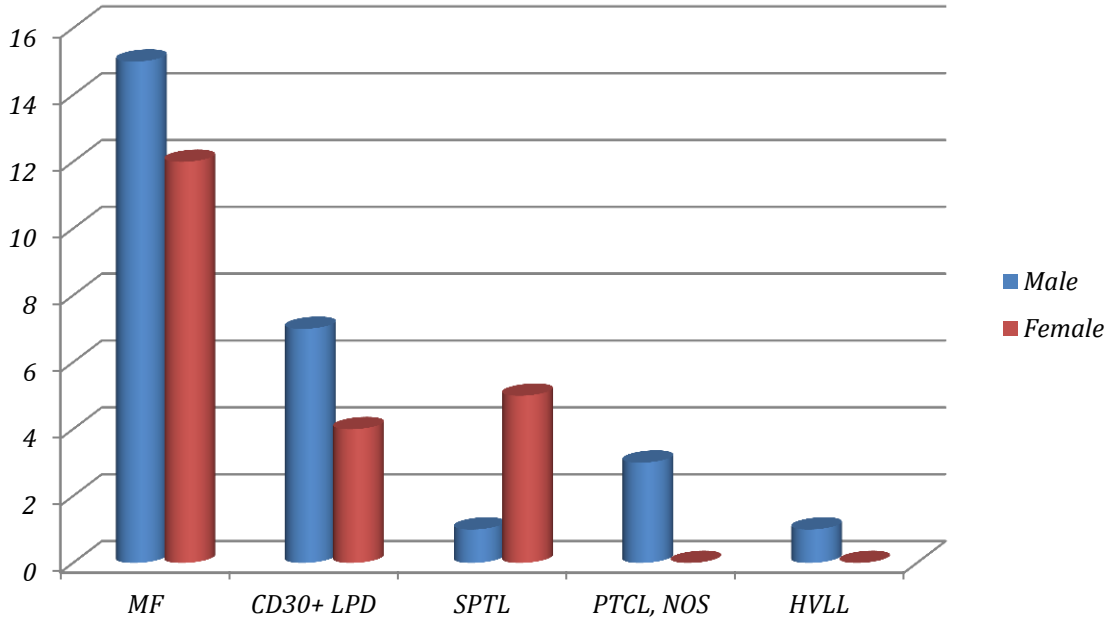


Figure 3. Gender distribution in primary CTCL

Table 7. Primary CTCL –Age at diagnosis and duration of disease prior to diagnosis

Primary CTCL (n=48)		MF (n=27)	CD30+ LPD (n=11)	SPTL (n=6)	PTCL, NOS (n=3)	HVLL (n=1)	*P*
Age at diagnosis	**Mean**	41.1±18.8	33.8±17.1	33.6±17.3	56.3±18.9	14	~
	Median	43	39	27.5	52	~	~
	Range	5-74	2-67	19-60	40-77	~	~
Duration of disease (in months)	**Mean**	92.7±94	35±58.5	17.8±32.5	4.6±1.5	12	~
	Median	72	12	6	6	~	< .001
	Range	6-400	4-204	0.5-84	3-6	~	~

Age at diagnosis and duration of disease [Table 7]

Mean age at diagnosis of primary CTCL was 38.9±18.7 years (range 2-77 years). SPTL was diagnosed at a younger age (median – 27.5 years) while PTCL, NOS was diagnosed at a later age (median- 52 years). Mean duration of skin lesions prior to diagnosis was 64 months (range 0.5 – 400 months, median - 24 months). Duration of the disease prior to the diagnosis of MF (median-72 months) was much longer than other subtypes of CTCL ($P < .001$). Median duration of disease prior to diagnosis of SPTL and PTCL, NOS was 6 months.

Table 8. Primary CTCL - Comparative clinical profile of each subtype of primary CTCL

Primary CTCL (n=48)		MF (n=27)	CD30+ LPD (n=11)	SPTL (n=6)	PTCL, NOS (n=3)	HVLL (n=1)
Morphology (n, %)	**Papules**	0	9/11, 81.8%	~	~	1, 100%
	Patches	23/27, 85.2%	~	~	~	~
	Plaques	11/27, 40.7%	~	1/6, 16.7%	2/3, 66.7%	~
	Nodules/tumors	5/27, 18.5%	3/11, 27.3%	6/6, 100%	3/3, 100%	~
	Erythroderma	2/27, 7.4%	1/11, 9.1%	~	~	~
	Polymorphic	9/27, 33.3%	2/11, 18.2%	2/6, 33.3%	2/3, 66.7%	~
Area of involvement	**Head and neck**	4/27, 14.8%	1/11, 9.1%	~	3/3, 100%	1, 100%
	Trunk	25/27, 92.6%	7/11, 63.6%	6/6, 100%	2/3, 66.7%	
	UL	25/27, 92.6%	8/11, 72.7%	3/6, 50%	2/3, 66.7%	1, 100%
	LL	27/27, 100%	7/11, 63.6%	4/6, 66.7%	2/3, 66.7%	1, 100%
	Multifocal	27/27, 100%	4/11, 36.4%	4/6, 66.7%	2/3, 66.7%	1, 100%
B-symptoms (n, %)		2 (7.4%)	1/11 (9.1%)	4/6, (66.7%)	1/3 (33.3%)	1 (100%)
Extracutaneous involvement (n, %)		4 (14.8%)	0	0	0	0

H- Head and neck; T- Trunk; UL- Upper limbs; LL- Lower limbs; Duration of disease was calculated in months;

Type of skin lesions and area of involvement [Table 8]

Majority of the patients with CD30+ LPD presented with papules (81.8%) while patches and nodules were the most common morphological skin lesions in MF and SPTL respectively. Three patients presented with erythroderma, of whom 2 had MF and one had cutaneous anaplastic large cell lymphoma. Most common area of involvement in MF was the lower limbs while trunk was the most area of involvement in SPTL. All three patients with PTCL, NOS had involvement of head and neck region.

Mycosis Fungoides

Frequency, gender and age distribution

MF was the most common CTCL and accounted for 56.25% patients (n=27). MF was more common in males (M/F ratio 1.25). Four patients (14.8%) belonged to paediatric age group (M/F ratio 3:1). Mean age at presentation in the adult age group (>16 years) was 47.5 ± 15.5 years (range 22-74 years) while the same in paediatric age group (≤16 years) it was 11.5 ± 3.8 years (range 6-14 years).

Duration of disease prior to the diagnosis

Mean duration of skin lesions prior to diagnosis of the disease was 92.7 ± 93.9 months (range 6-400 months, median 72 months) [**Table 7**]

Mean duration of skin lesions after excluding the two extreme variables (400 & 300 months) from the data was 72.2 ± 58.3 months (range 6-220 months, median 72 months).

Types of skin lesions

Patches were the most common morphological type of skin lesion accounting for 85.2% (n=23) followed by plaques (11, 40.7%), tumours (5, 18.5%) and erythroderma in two patients (7.4%) while ulceration of existing skin lesions was noted in 3 patients (11.1%) [**Table 8**]

Hypopigmented skin lesions were noted in 5/27 (18.5%) patients. One patient (3.7%) had vesicular lesions on palms and soles. Poikilodermatous MF was noted in 7 patients (25.9%). Patches and plaques coexisted in 4 patients (14.8%) and all three lesions (patches, plaques and tumours) coexisted in 5 patients (18.5%). [**Figure 4**]

In the paediatric age group 3/4 children (75%) presented with patches, 1/3 children had hypopigmented patches and 1/4 children (25%) presented with plaques.

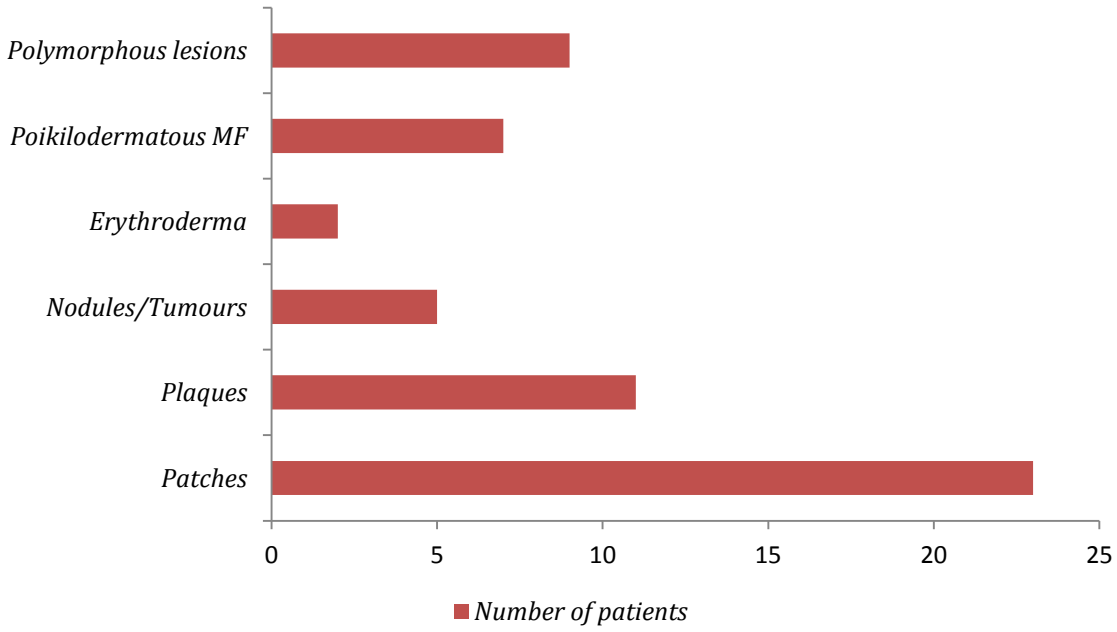


Figure 4. Morphology of skin lesions in MF

Anatomical distribution of skin lesions [Table 8]

All patients had more than one anatomical area of involvement. The lower limbs were involved in all patients. Skin lesions on trunk and upper limbs were noted in 92.6% (n=25) patients and involvement of head and neck was noted in 51.8% (n=14) patients [**Figure 5**]. Palms and soles were involved in 4 patients (14.8%). Extent of involvement varies from 5% to > 90% of body surface area.

All patients in the paediatric age group had skin lesions involving >1 anatomical area except one patient who had skin lesions only in one area. Most common anatomical area of involvement in the paediatric age group were lower limbs (4, 100%) followed by face (3, 75%), upper limbs (3, 75%) and trunk (2, 50%). Body surface involvement of skin lesions in children varied from 5% - 50%.

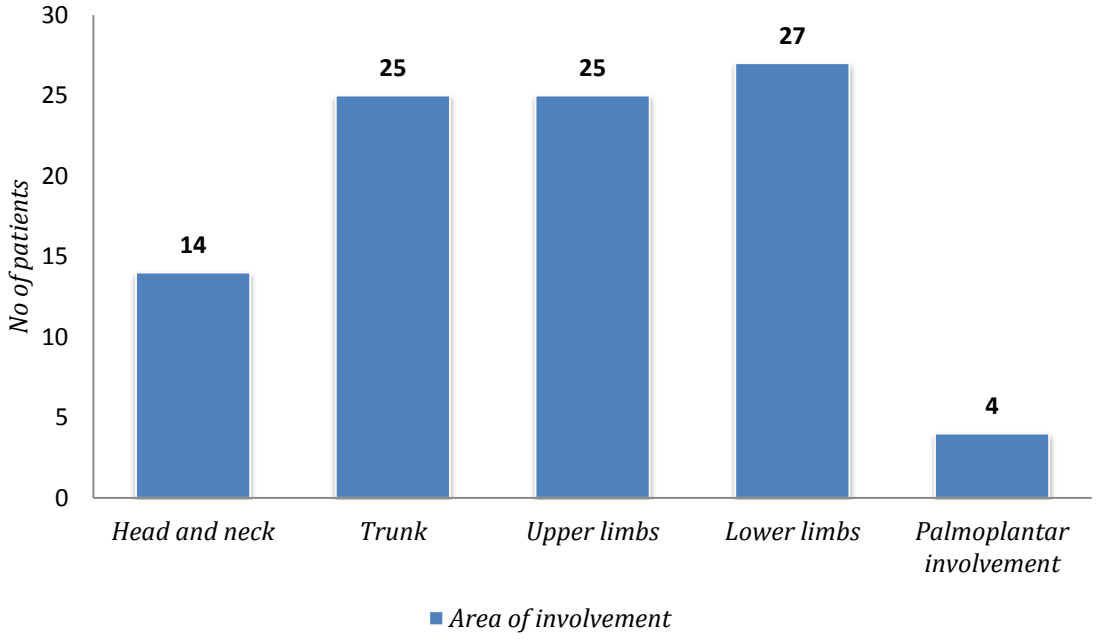


Figure 5. Anatomical area of involvement in MF

'T' stage of skin lesions

Most of the patients presented in T2a stage (14, 51.8%) followed by T2b (5, 18.5%), T3 (4, 14.8%) and T4 (2, 7.4%). [**Figure 6**] In the paediatric age group 3 patients (75%) were in T2a stage and one patient (25%) was in T1b stage.

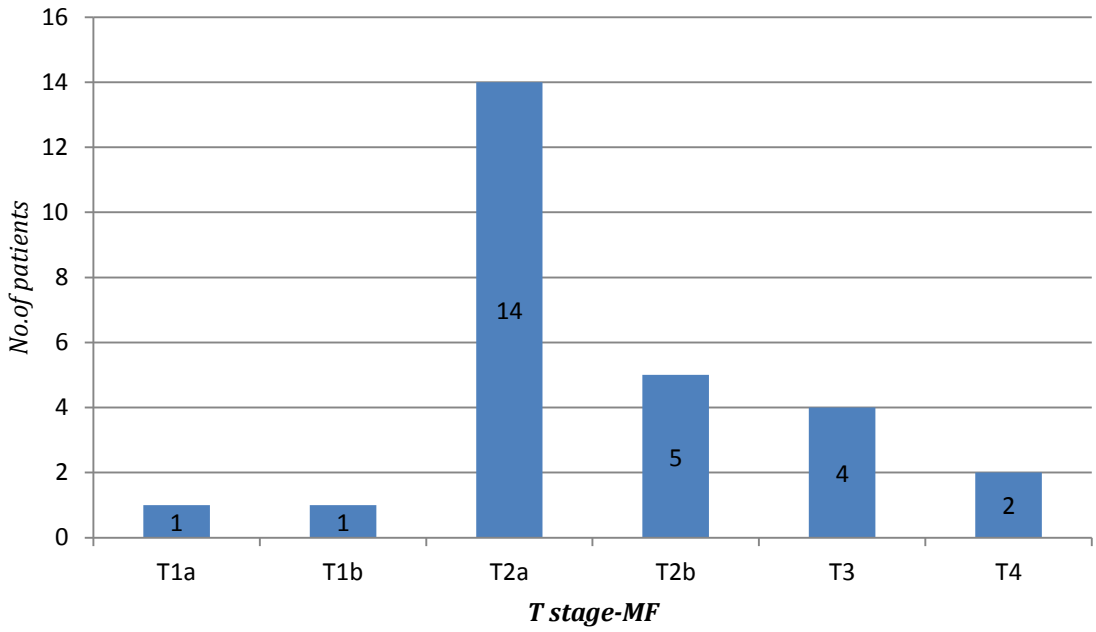


Figure 6. Frequency of patients at different stages of MF

B-symptoms

Two patients (7.4%) presented with significant weight loss and one patient additionally (50%) had prolonged fever. None of the children diagnosed as MF had any systemic symptoms at the time of presentation.

Histopathological features [Table 9]

Epidermotropism was noted in 25 patients (92.6%) and Pautrier's microabscesses were seen in 9 patients (33.3%). Superficial dermal atypical lymphoid infiltrates (including both perivascular and lichenoid band like infiltrates) was noted in

25.9% (n=7) patients. Atypical lymphoid infiltrates extended to deep dermis in 5 patients (18.5%) among which 60% (n=3) patients belonged to stage T3 and T4. Involvement of subcutis was noted in 2 patients (7.4%) of T3 stage of MF. Large cell transformation was noted in 2 patients (7.4%) among which one patient belonged to T3 stage and the other patient belonged to T4 stage. Other features like lymphocyte tagging at dermoepidermal junction was seen in 18 patients (66.6%), mild to moderate spongiosis in 9 patients (33.3%) and eosinophilia in 2 patients (7.4%).

Table 9. Relative frequency of salient histological features of MF

T stage	Epidermotropism	Pautrier's microabscess	Extension of atypical infiltrates to deep dermis and subcutis	Large cell transformation
T1a (n=1)	1/1(100)	0	0	~
T1b (n=1)	0	1/1(100)	0	~
T2a (n=14)	14/14(100)	3/14(21.4)	1/14 (7.1)	~
T2b (n=5)	4/5 (80)	2/5(40)	1/5 (20)	~
T3 (n=4)	4/4(100)	2/4(50)	4/4 (100)	1/5 (20)
T4 (n=2)	2/2(100)	1/2(50)	1/2 (50)	1/2 (50)
Total (n=27)	25/27 (92.6)	9/27(33.3)	7/27 (26)	2/27 (7.4)

( ) In percentage; 'T' staging based on ISCL/EORTC revised clinical staging and classification of mycosis fungoides and Sezary syndrome

Immunohistochemistry [Table 10]

Pan T-cell marker CD3 was positive in all patients of MF. CD4 positivity was noted in 100% patients (n=27). CD8>CD4 was noted in 3/27 patients (stage T2a). Two adults and one child were the three patients with predominant CD8 positivity. Aberrant loss of CD7 antigen was noted in 25 patients (92.5%) and CD30 positivity in 2 patients (7.4%). Mib-1 index was done in 8/27 patients (1/14 patient in stage T2a, 2/5 patients in stage T2b, 3/4 patients in stage T3 and 2/2 patients in stage T4) among which 50% (n=4) (2 patients in stage T3 and one each in stage T4 and stage T2b) had index more than 30%.

Table 10. Immunohistochemical features of MF (n=27)

T stage	CD3+	CD4+	CD7-	CD30+	CD8>CD4	MIB index	
						<30%	>30%
T1a (n=1)	1/1	1/1	1/1	0	~	ND	ND
T1b (n=1)	1/1	1/1	1/1	0	0	ND	ND
T2a (n=14)	14/14	14/14	14/14	0	3/14	1	~
T2b (n=5)	5/5	5/5	4/5	0	0	1	1
T3 (n=4)	4/4	4/4	3/4	2/4	0	1	2
T4 (n=2)	2/2	2/2	2/2	0	0	1	1
Total (n=27)	27/27	27/27	25/27	2/27	3/27	4	4

ND – Not done

Bone marrow biopsies [Table 11]

Bone marrow biopsy was done in 9/27 patients (33.3%). This included 2/14 patients in stage T2a, 2/5 in stage T2b, 3/4 patients in stage T3 and 2 in stage T4. 3/9 patients (1 each in stage T2b, T3 and T4) (33.3%) had involvement of the bone marrow.

Lymph node biopsies [Table 11]

Five patients (18.5%) had lymph node enlargement. Lymph node biopsy was done in 3/27 patients (11.1%) (2 patients were in stage T4 and one patient in stage T2a) who had significant enlargement of lymph nodes. One patient (33.3%) in stage T4 had involvement of lymphoma in the lymph node.

Laboratory parameters [Table 11]

ESR was done in 11/27 patients (40.7%) of whom 2 patients (18.1%) had elevation of ESR. One patient (3.7%) had Sezary cells in the peripheral smear. Hypoalbuminemia (serum albumin < 3.5 gm/dl) was noted in 1/23 patients (4.34%) and there was elevation of transaminases in 1/23 patients (4.35%). Serum alkaline phosphatase was elevated in 4/23 patients (17.3%) among whom one was a child and three were adults.

Serum lactate dehydrogenase (Normal range 0-460 IU/l) was done in 23 (85.2%) patients of which 10/23 patients (43.5%) had elevated LDH. A patient in stage IIIB had LDH level more than 1000 IU/l.

Viral markers

Viral markers including HIV, hepatitis-B and hepatitis-C were done in 12 patients (44.4%) and all of them were negative for the same. HTLV-1 antibody was found positive in an erythrodermic MF patient.

Table 11. Primary CTCL - Laboratory parameters, imaging studies, bone marrow, lymph node biopsy and organ biopsy

Primary CTCL (47)	LDH	CXR	Abnormality in USG	Other imaging	BM involvement	Abnormal LN B_x	Misc B_x
MF (27)	10/23 (43.5%)	0/20	0/17	1/8 (12.5%)	2/9 (22.2%)	1/3 (33.3%)	0/1
cALCL (3)	2/3 (66.7%)	0/3	0/2	0/1	0/3	0	0
LyP (8)	2/7 (28.6%)	0/7	0/7	0	0/1	0	0
SPTL (6)	5/6 (83.3%)	0/6	0/5	0/3	0/6	0	0/1
PTCL, NOS (3)	2/3 (66.7%)	0/3	0/2	0/1	0/2	0	0
HVLL (1)	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1	0/1	0/1	0/1
Total	43 (22, 51.1%)	1/40 (2.5%)	1/34 2.9%)	1/14 (7.14%)	2/22 (9.1%)	1/4 (25%)	0/3

LDH - Lactate dehydrogenase; CXR – Chest X-ray; USG- Ultrasonography of abdomen and pelvis; BM – Bone marrow; LN B_x – Lymph node biopsy; Misc B_x – Miscellaneous organ biopsy; Other imaging includes CT scan, MR imaging and PET- CT scan; Denominator indicates the number of patients in whom the test was done

Imaging studies [Table 11]

Chest X-ray

Chest X-ray was done in 20 patients (74.1%) and none of them were detected with disease specific abnormalities.

Ultrasonography

Ultrasound imaging of the abdomen and pelvis was done in 17 patients (63%) and none of them were detected with any disease specific abnormality.

Computed tomography imaging (CT scan)

Computed tomography imaging of thorax was done in 6 patients (7.4%) and CT imaging of the abdomen was done in 4 patients (14.8%). None of them were detected with disease specific changes.

Positron emission tomography

Two patients (7.4%) underwent positron emission tomography imaging of whole body; one patient (50%) had extracutaneous (nodal) involvement the disease.

Extra-cutaneous involvement

Among 4/27 (14.8%) patients with extracutaneous involvement, 2 (50%) were in T4 stage while one (25%) was in T3 stage and one (25%) was in T2b stage. 3/4 (75%) patients had bone marrow involvement and 1/4 (25%) patients had extracutaneous nodal involvement.

TNM staging

Complete staging was done in 22/27 patients who were screened by either of the imaging studies (22/27) (Ultrasonography, CT scan, MRI scan or PET scan), lymph node biopsies (3/27) and bone marrow biopsies (9/27). As 5/27 patients had only

limited skin involvement (4 patients in stage T2a and 1 patient in stage T1b) and normal physical examination, neither imaging studies nor organ biopsies were done in them. Most of the patients belonged to stage IB (18, 66%) followed by 3 patients (11%) in stage IA, 3 patients (11%) in stage IIB and each in stage II, stage IIIB and IVA2 [**Table 12].**

Table 12. TNM staging of MF

TNM staging	No of patients	Frequency (%)
IA	3/27	11.1
IB	18/27	66.7
II	1/27	3.7
IIB	3/27	11.1
IIIB	1/27	3.7
IVA2	1/27	3.7

CD8 positive MF

Three patients (2 adults and 1 child)showed predominant CD8 positivity in skin. All three patients presented with hypopigmented macules on the trunk and lower limbs. None of the patients had any systemic or laboratory abnormalities. All three patients belonged to 'T2a' stage of the disease.

Molecular study (TCR gene rearrangement assay)

In 22 patients with patch/plaque (Stage I/IIA) mycosis fungoides, the histological diagnosis of mycosis fungoides could not be excluded or the diagnosis was suggestive but not confirmatory in 14 patients. TCR gene rearrangement assay was done in 13/14 patients. Among them, 11/13 patients (84.6%) belonged to T1a/T2a stage of MF and 2/13 patients (15.4%) belonged to T2b stage. One patient belonged to T3 stage of disease. Monoclonality was detected in 63.6% (7/11) patients of patch stage MF (T1a/T2a), 50% (1/2) patients of T2b stage. Monoclonality was detected in the patient with T3 stage. **[Table 13]**

Table 13. Clonal detection rate in MF

MF stage	TCR gene rearrangement assay			
	Monoclonal (n=9)		Polyclonal (n=5)	
	No	Frequency (%)	No	Frequency (%)
T1a/T2a (11)	7	63.6%	4	36.4%
T2b (2)	1	50%	1	50%
T3 (1)	1	100%	0	
Total (14)	9	64.3%	5	35.7%

( ) No. of patients in each 'T' stage of MF

Histopathological features in monoclonal and polyclonal patients

Histological diagnosis of MF was classified based on histological criteria for diagnosis of MF proposed by Guitart et al (35). Histological diagnosis of early stage MF (I/IIA) was compared with clonal detection rate [**Table 14**]. Monoclonality was detected in 80% (n=4) of patients in whom the histology was categorised as

probable/suggestive of MF versus 50% (n=4) of patients in whom it was categorised as MF cannot be excluded. Loss of lineage of T-cell differentiation antigens in early MF was found in 6/8 (75%) patients in monoclonal group while 2/5 (40%) showed the same in polyclonal group [**Table 14**]. One patient with CD8 positivity showed monoclonality and the other patient with CD8 positivity showed polyclonal pattern.

Table 14. Relationship between histopathological diagnosis and clonality in early stage (I/IIA) MF

Histological and immunohistochemical diagnosis of MF [Categorised as per grading protocol proposed by Guitart et al] (35)	**Monoclonal (n=8)**		**Polyclonal (n=5)**		***P***
	Number	%	Number	%	
MF cannot be excluded (n=8)	4/8	50%	4/8	50%	0.564
Probable/suggestive of MF (n=5)	4/5	80%	1/5	20%	
CD3+ , CD4+ and CD7-	6/8	75%	2/5	40%	0.2929
CD8>CD4	1/8	12.5%	1/5	20%	

CD30 positive lymphoproliferative disorders (CD30+ LPD)

Frequency and gender distribution

Eleven patients (7 males, 4 females) had CD30+ LPD of whom 8 patients had lymphomatoid papulosis (72.7%) and 3 patients (27.3%) had primary cutaneous ALCL (cALCL). The M/F ratio was 1.75 [**Figure 7]**.

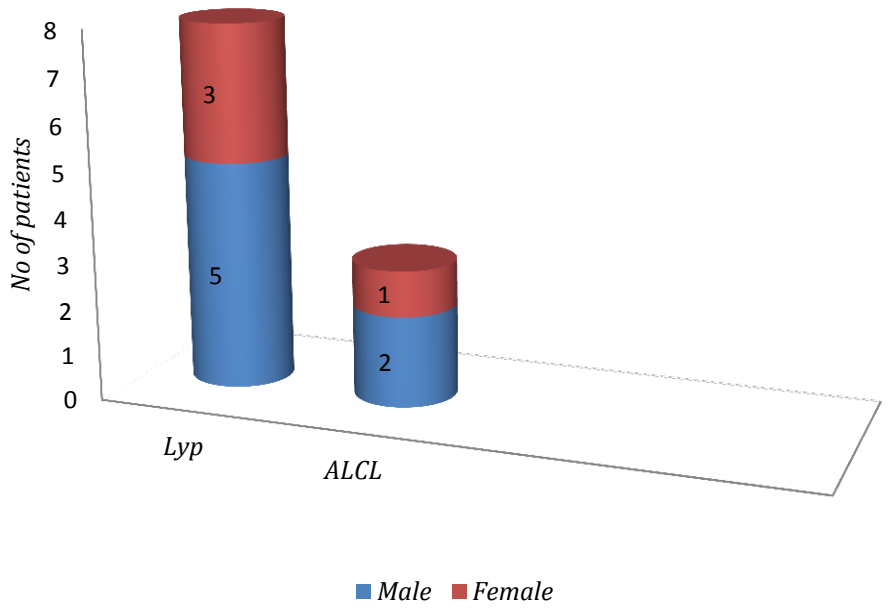


Figure 7. Relative frequency and gender distribution in primary CD30+ LPD

Age distribution

Mean age of presentation was 34.6 ± 17.9 years (range 2-70 years). One patient was a female child aged 2 years and rest were adults (>16 years).

Age at diagnosis

Mean age at diagnosis of the disease was 33.8 ± 17.1 years (range 2-67 years)

Duration of disease [Table 7]

Mean age at which the patients noticed the skin lesions prior to the diagnosis was 30.9 ± 17.9 years (range 1-65 years). Median duration of skin lesions prior to the diagnosis of the disease was 12 months (range 4-204 months, mean 35 ± 58.5 months).

Types of skin lesions [Table 8]

Papules were the most common morphological skin lesion noted in 9 patients (81.8%), followed by plaques in 2 patients (18.2%) and nodules in 2 patients (18.2%). One patient (9.1%) each presented with erythroderma and noduloulcerative skin lesions.

Anatomical distribution [Table 8]

Upper limbs were the most common anatomical area of involvement followed by lower limbs and trunk. 72.7 % (n=8) had involvement of upper limbs while 63.6 % (n=7) had skin lesions on trunk and lower limbs. Involvement of head and neck was seen in one patient (9.1%). Seven patients (63.6%) had skin lesions in more than one anatomical area and four patients (36.4%) had skin lesions only in one anatomical area. Except one patient who presented with erythroderma rest of the patients had less than 10% of body surface involvement.

Past treatment

2/9 (22.2%) patients in the past were treated with systemic chemotherapy.

Lymphomatoid papulosis (LyP)

Frequency and gender distribution

Eight patients (72.7%) had LyP among which 5 were males and 3 were females (M/F 1.66)

Age at diagnosis [Table 7]

Mean age at diagnosis of disease was 28.25±14.7 years (range 2-44 years) and all the patients were newly diagnosed during the study period.

Duration of disease [Table 7]

Median duration of skin lesions prior to the diagnosis was 12 months. Mean duration of skin lesions prior to the diagnosis was 43.1±67.7 months (range 6-36 months).

Types of skin lesions [Table 8]

All the patients presented with papules while one patient had coexistent noduloulcerative skin lesions.

Anatomical distribution [Table 8]

37.5 % (n=3) of patients had skin lesions on trunk and both extremities while 25% (n=2) had involvement of upper and lower limbs. 37.5% (n=3) of patients had single anatomical area of involvement. None of the patients had skin lesions on head and neck. Extent of involvement was less than 10% in all patients.

Systemic involvement

One patient presented with fever for more than a month. None of the other patients presented with any systemic symptoms. Neither of the patients presented with lymph node enlargement nor with extracutaneous organ involvement.

Laboratory investigations [Table 11]

LDH level was elevated in 2/7 patients. None of the patients had anaemia or other cytopenia.

Histopathological features [Table 15]

All 7 patients were classified from Type A-D based on histological features. The most common type was LyP type A (5, 62.5%). One patient had an unusual pattern

(Type D) which histologically simulates an aggressive cytotoxic lymphoma but clinically indolent in nature. Epidermotropism was noted in 2/8 patients (25%) and tagging of lymphocytes along dermoepidermal junction was noted in 3/8 patients (37.5%). Atypical lymphocytic infiltrates extended upto subcutis in 1/8 (12.5%) patients while it extended upto deep dermis in 4/8 patients (50%). Mixed inflammatory infiltrates in dermis was noted in 5 patients and increased mitotic activity was evident in 5 patients.

Table 15. Histological types of LyP

Histological types	LyP (n=8)	
	No	Freq
Type A	5/8	62.5%
Type B	2/8	25%
Type C	0/8	0
Type D	1/8	12.5%

No - Number of patients; Freq - Frequency

Immunohistochemical profile [Table 16]

Diffuse CD30 positivity was noted in 3/8 patients (37.5%)and CD30 was focally positive in 5/8 patients (62.5%). Pan T-cell marker (CD3) was positive in 6 patients while CD4 was positive in 5 patients and CD8 was positive in 2 patients. One patient showed positivity of granzyme B and TIA-1 antigen. CD56 marker was done in 2 patients of whom one showed positivity of the same. Mib-1 index was done in 5 patients (62.5%) and it was <10% in 3 patients, 10-30% in 1 patient and >30% in 1 patient.

Table 16. Immunohistochemical markers pattern in CD30+ LPD

IHC markers		LyP (n=8)		cALCL (n=3)	
		Number of patients	Frequency (%)	Number of patients	Frequency (%)
CD30+ (n=8)	**Focal**	5/8	62.5	0/3	~
	Diffuse	3/8	37.5	3/3	100
CD3+		6/8	75	3/3	100
CD4+		5/8	62.5	2/3	66.7
CD8+		2/8	25	0/3	~
CD7-		4/8	50	~	~
ALK negative		1/1	100	3/3	100%
Mib-1 index	**<10%**	3/5	60	1/2	50
	>10%	2/5	40	1/2	50

ALK – Anaplastic lymphoma kinase;

Bone marrow biopsy

Bone marrow biopsy done in 1/8 patient (14.3%) (Stage IIB) was normal.

Imaging studies [Table 11]

Chest X-ray and ultrasound imaging of abdomen was done in all 8 patients and none of them had any disease specific abnormalities.

Molecular study (TCR gamma gene rearrangement study)

TCR gamma gene rearrangement was done in one patient (Type B LyP) who showed a dominant T-cell clone.

Staging of lymphomatoid papulosis [Table 17]

Based on 'T' stage of skin lesions, four patients (50%) belonged to stage T3b while 1 patient each belonged to stage T1a, T2a, T2b and T3a respectively. Based on TNM classification, 5 patients belonged to stage IIB while 2 patients belonged to stage IB and 1 patient belonged to stage IA. **[Table 17**]

Table 17. TNM staging in LyP and cALCL

TNM stage	LyP (n=8)		cALCL (n=3)	
	No of patients	Frequency	No of patients	Frequency
IA	1	12.5%	0	~
IB	2	25%	2	66.7
IIB	5	62.5%	1	33.3

Primary cutaneous anaplastic large cell lymphoma (cALCL)

Frequency and Gender distribution

Three patients (2 males and 1 female) had primary cutaneous anaplastic large cell lymphoma.

Age distribution

All the patients were adults more than 40 years of age. Mean age at presentation was 51.7±15.9 years (range 41-70 years).

Duration of skin lesions

Mean duration of skin lesions prior to the diagnosis was 13.3±10.1 months.

Course of disease

Two patients presented with relapse of the disease after completion of treatment with systemic chemotherapy (Cyclophosphamide, doxorubicin, vincristine and prednisone based chemotherapy). In one patient the disease had relapsed 4 years after completion of treatment and in another patient the disease relapsed within a year after treatment.

Morphology of skin lesions

The patients presented with papulonodular lesions (1, 33.3%), noduloulcerative skin lesions (1, 33.3%) and erythroderma (1, 33.3%).

Anatomical distribution and extent of involvement

The lesions were confined to trunk in 1 patient (33.3%) and both trunk and upper limbs were involved in 1 patient (33.3%). Extent of involvement was < 10 % in 2 patients and one patient had >80% involvement.

Systemic involvement

One patient each had peripheral lymph node enlargement (axillary and inguinal groups) and mild hepatosplenomegaly.

Laboratory investigations

Mild anaemia and hypoalbuminemia was noted in one patient (33.3%).LDH level was elevated in 2 patients (66.7%). LDH level was >1000 IU/L in the patient with erythrodermic ALCL. The same patient also had atypical lymphocytes (13%) in the peripheral blood.

Histopathological features in primary cALCL [Table 18]

Increased mitotic activity was noted in 2/3 patients (66.7%) and mixed inflammatory infiltrates in 2/3 patients (66.7%). Multinucleated cells and extensive neutrophilic infiltrates were noted in 1 patient (33.3%)

Bone marrow biopsy

Bone marrow biopsy did not show any evidence of involvement with lymphoma in all three patients.

Table 18. Histological features in primary cALCL

Histological features in ALCL	Patient 1	Patient 2	Patient 3
Size of atypical cells	Medium to large	Large	Medium
Depth of atypical cell infiltrates	Superficial dermis	Deep dermis	Superficial dermis
Inflammatory infiltrates	Absent	Present	Present
Mitoses	Present	Absent	Present
Multinucleated cells	Absent	Present	Absent
Bone marrow biopsy	Normal	Normal	Normal

Immunohistochemical features [Table 16]

Diffuse CD30 positivity was noted in all three patients while ALK was negative in all three patients. Pan T-cell marker CD3 was positive in all patients and B-cell marker CD20 was negative. CD4 positivity was seen in 2 patients (66.7%) while none of them showed CD8 positivity. Mib-1 index was <10% in 1 patient and >30% in 1 patient.

Imaging studies

Chest X-ray and ultrasound imaging of abdomen did not show any disease specific abnormality.

Staging of primary cALCL [Table 17]

One patient had extracutaneous nodal involvement. Based on extent of involvement of skin lesions the patients were staged as T3b, T2b and T2a each. As per TNM classification the patients were staged as $T_{3b}N_1M_0$ (IIB), $T_{2b}N_0M_0$ (IB) and $T_{2a}N_0M_0$ (IB) [**Table 17**].

Subcutaneous panniculitis like T-cell lymphoma (SPTL)

Age and gender distribution

All patients diagnosed with SPTL were >16 years. There were 5 females and a male. (M/F 1:5).

Age at diagnosis

Mean age at diagnosis of the disease was 33.7±17.2 years (range 19-60 years, median 27.5 years).

Duration of skin lesions [Table 7]

Median duration of skin lesions prior to the diagnosis of the disease was 6 months (Mean – 17.8 ± 32.5 months, range – 0.5-84 months).

While 4 patients (66.7%) were newly diagnosed, one patient was in remission after treatment and one patient presented with relapse of the disease. Relapse of the disease occurred 3 years after the completion of systemic chemotherapy.

Types of skin lesions [Table 8]

All six patients (100%) presented with subcutaneous nodules and one patient presented with large purpuric macules overlying the subcutaneous indurated nodules. In one patient (16.6%) plaques coexisted with nodular skin lesions.

Anatomical distribution [Table 8]

Most common area of involvement was trunk (5, 83.3%) followed by lower limbs (4, 66.6%) and then upper limbs (3, 50%). Involvement of face was noted in 2 patients (33.3%). Four patients (66.7%) had involvement of >1 anatomical area. Total body surface area involvement of skin lesions was <10% in 5 patients (83.3%) and one patient had involvement of >10%.

Systemic involvement

Four patients (66.7%) presented with systemic symptoms including fever, weight loss and pedal edema. 50% (n=3) patients had peripheral lymph node enlargement. Two patients (33.3%) had hepatomegaly among which one patient had ascites.

Laboratory investigations [Table 11]

Four patients (66.7%) had anaemia (Haemoglobin <12 gm/dl); one patient (16.6%) had mild thrombocytopenia (<1 lakh cu.mm) and one patient had elevated

ESR. Hypoalbuminemia was noted in 3 patients (60%) and elevated transaminases were noted in 2 patients (40%). LDH level was elevated in 5 patients (83.3%) and it was more than 1000 IU/l in 3 patients (50%). Viral markers (HIV, hepatitis-B and hepatitis- C) done in all patients were negative. EBV PCR done in a patient was negative.

Histopathological features [Table 19]

Unique histopathological features noted in all patients of SPTL were atypical lymphoid infiltrates involving subcutis and rimming of adipocytes by atypical lymphocytes. Predominant lobular panniculitis was noted in all patients while septal involvement was noted in one patient (16.7%). Fat necrosis was noted in 2 patients (33.3%) and haemophagocytosis was noted in 1 patient (16.7%).

Immunohistochemical features [Table 19]

Pan T-cell marker (CD3) and cytotoxic T-cell marker (CD8) were positive in all patients. Three patients showed focal positivity of CD4. Granzyme B done in 4/6 (66.7%) patients showed positivity of the same and CD56 done in 5/6 (83.3%) patients was negative. TIA-1 antigen done in 3/6 (50%) showed positivity of the same. Mib-1 index was 11-30% in 4/6 patients (66.7%) and >30% in 2/6 patients (33.3%).

Table 19. Histological and immunohistochemical features of SPTL

Subcutaneous panniculitis like T-cell lymphoma		Number of patients (n=6)	Frequency
Panniculitis	**Lobular**	6/6	100%
	Septal	1/6	16.7%
Fat necrosis		2/6	33.3%
Haemophagocytosis		1/6	16.7%
CD4+		3/6	50%
Granzyme B		4/6	66.7%
CD56 negativity		5/6	83.3%
TIA-1 antigen positivity		3/6	50%
Mib-1 index	**<50%**	4/6	66.7%
	>50%	2/6	33.3%

TIA-1 – T-cell intracellular antigen -1

Bone marrow biopsy

Bone marrow biopsy was done in all six patients and there was no evidence of lymphoma involvement in bone marrow.

Imaging studies [Table 11]

Chest X-ray

Chest X-ray was normal in all patients.

Ultrasonography

Ultrasound imaging of abdomen was done in 5/6 (83.3%) patients and none of them showed any disease specific abnormalities.

CT scan

CT scan of thorax and abdomen done in 3 patients did not show any disease specific abnormality.

PET-CT scan

PET-CT scan done in a patient did not show any features of extra cutaneous involvement.

Extracutaneous involvement

None of the patients had extracutaneous involvement at the time of presentation.

Staging of SPTL [Table 20]

Three patients were in stage T3b while 2 patients were in stage T3a and 1 patient was in stage T2b. Based on TNM staging, four patients (80%) were in stage IIB while two patients were in stage IB.

Table 20. 'T' stage and TNM stage in SPTL patients

Staging		Number of patients (n=6)	Frequency
T staging	T2b	1/6	16.7%
	T3a	2/6	33.3%
	T3b	3/6	50%
TNM staging	IB	2/6	33.3%
	IIB	4/6	66.7%

Peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS)

Frequency and gender distribution

PTCL, NOS constituted 6.25% (n=3) of all primary CTCLs. All the three patients were males and were newly diagnosed during the study period.

Age distribution and duration of skin lesions [Table 6]

All 3 patients were adults (>16 years). Mean duration of skin lesions prior to the diagnosis was 4.7±1.5 months (range 3-6 months).

Age at diagnosis

Mean age at diagnosis of the disease was 56.3 ± 18.9 years (range 40-77 years).

Types of skin lesions [Table 8]

All 3 patients presented with nodules, in 1 patient they were ulcerated. Two patients (66.7%) had plaques coexisted with nodules

Anatomical distribution [Table 8]

One patient (33.3%) had a solitary nodular skin lesion on face and rest (2, 66.7%) had skin lesions involving face, trunk and both extremities

Systemic symptoms

None of the patients had any significant systemic symptoms at the time of presentation. One patient had peripheral lymph node enlargement.

Laboratory investigations [Table 11]

One patient (33.3%) had thrombocytopenia and 2 patients (66.7%) had elevated ESR values. Hypoalbuminemia was noted in one patient. All the patients had normal liver enzymes. LDH was elevated in 2 patients (66.7%). Viral markers (HIV, hepatitis-B and hepatitis- C) done in all patients were negative.

Histopathological features

Two patients (66.7%) had atypical lymphoid infiltrates involving the whole dermis (pandermal) and one patient (33.3%) had atypical lymphoid infiltrates extending to the mid dermis. One patient (33.3%) displayed epidermotropism and another patient showed lymphocyte tagging along the dermoepidermal junction.

Immunohistochemical markers [Table 21]

Pan T-cell marker (CD3) and helper T-cell marker (CD4) were positive in all three patients while cytotoxic T-cell marker was positive in 2 patients (66.7%). In all three patients ratio of CD4 positive cells were more than CD8 positive cells. Aberrant loss of CD7 antigen is noted in all three patients. Other immunological markers including granzyme B, TIA-1and CD56 were negative in 2 patients. CD30 was negative in all 3 patients. Mib-1 index was <10% in 1 patient, >30% in the rest of the 2 patients. EBV marker done in 1 patient was negative.

Bone marrow biopsy

Bone marrow biopsy was done in two patients and none of them showed involvement of bone marrow.

Imaging studies [Table 11]

Chest X-ray

Chest X-ray was normal in all 3 patients.

Ultrasonography

Ultrasound imaging of abdomen was done in 2 patients (66.7%) who did not show any involvement of other organs or central lymph nodes.

PET scan

PET scan done in a patient did not show features of any extracutaneous involvement.

Staging of PTCL, NOS

None of the patients had extracutaneous involvement at the time of presentation. Two patients were in stage T3b and one patient was in stage T1a. Based on TNM staging, 1 patient was in stage IA and two were in stage IIB.

Clinical, histological and immunological features of all three patients of PTCL, NOS are summarised in **Table 21.**

Table 21. Clinical, histological and immunohistochemical pattern in PTCL, NOS

Features	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Male
Age at diagnosis (in years)	52	40	77
'T'stage	T1a	T3b	T3b
Stage	IA	IIB	IIB
Skin lesions	N	P, N, U	P, N
Area involved	H	H, T, UL, LL	H, T, UL, LL
Extent of involvement	1%	>50%	30-50%
Extracutaneous involvement	No	No	No
LDH (IU/L)	385	700	733
Immunological markers	CD3+, CD4+, CD8+, CD7-, CD56-, CD30- grB- and TIA1-	CD3+, CD4+, CD8+, CD7-, CD56-, CD30- grB- and TIA1-	CD3+, CD4+, CD8-, CD7- and CD30-

P- Plaque; N-Nodule; T- Trunk; U-Ulcer; H- Head and neck; T-Trunk; UL-Upper limbs; LL- Lower limbs; grB- Granzyme B; TIA-1- T-cell intracellular antigen-1; Staging was done based on ISCL/EORTC proposal on TNM classification of cutaneous lymphoma other than MF/SS

Hydroa vacciniforme like T-cell lymphoma (HVLL)

Hydroa vacciniforme like T-cell lymphoma, an EBV+ T-cell lymphoproliferative disorder of childhood occurred in a male child aged 14 years. Frequency of HVLL of all primary CTCL was 2.08%. Skin lesions were noticed since 1 year before the diagnosis was made.

Type of skin lesions and duration of disease

The patient had papulovesicular skin lesions of 1 year duration. Face, upper limbs and lower limbs were involved. Extent of involvement was <10% of total body surface area. He presented with facial edema of 1 year duration.

Systemic symptoms

The patient had low grade fever for 1 year. He also had weight loss for more than 6 months.

Laboratory investigations

Patient had hypoalbuminemia along with elevation of liver enzymes and alkaline phosphatase. LDH level was more than 1000 IU/L. Viral markers (HIV, hepatitis-B and hepatitis- C) were negative. Qualitative EBV PCR done was positive.

Histopathological and immunohistochemical features

Skin biopsy showed atypical lymphoid infiltrates which extended unto the underlying skeletal muscle. Immunohistochemistry profile was consistent with the diagnosis of hydroa vacciniforme like T-cell lymphoma. Atypical lymphoid infiltrates showed diffuse positivity for CD3, CD8, CD56 and granzyme B while it showed a focal positivity of TIA-1 antigen, CD30 and EBV LMP-1. CD20 marker was negative and there was aberrant loss of CD7 antigen.

Molecular study

TCR gamma gene rearrangement assay was done which showed dominant T-cell clonal population of cells.

Bone marrow, lymph node and liver biopsy

Bone marrow, lymph node and liver biopsies done did not show any features of systemic involvement.

Imaging studies

Ultrasound imaging of abdomen and pelvis revealed hepatosplenomegaly.

Staging of HVLL

'T' stage of the patient was T3b while the final TNM staging was $T_{3b}N_0M_0$ (IIB).

Primary cutaneous B-cell lymphoma

Diffuse large B-cell lymphoma, other

Clinical profile

A 65 year old male was diagnosed to have primary cutaneous B-cell lymphoma who presented with multiple erythematous annular plaques and underlying induration on the left forearm. Two years after onset of symptoms he had bone marrow involvement. Extent of involvement of skin lesions of the total body surface area was <5%. At presentation he did not have any systemic symptoms.

Laboratory investigations

All relevant investigations including haemoglobin, WBC count, platelets and ESR were normal. LDH level was normal (314 IU/L). Viral markers (HIV, hepatitis-B and hepatitis- C) done in the patient were negative.

Histopathological and immunohistochemical features

Skin biopsy from the plaque showed atypical lymphoid infiltrates extended till subcutis. Immunohistochemical profile was consistent with the diagnosis of T-cell rich diffuse large B-cell lymphoma. Both T-cell marker (CD3) and B-cell marker (CD20) were positive while CD30 marker was negative.

Bone marrow biopsy

Bone marrow biopsy done during initial diagnosis did not show any involvement. But it was found to be involved when the patient presented with a relapse involving the skin two years later.

Imaging studies

Chest X-ray done was normal. Ultrasound imaging of the abdomen and pelvis did not show any disease specific abnormality. Computed tomography imaging of the thorax was done which was normal.

Staging of PCLBCL, other

'T' stage of the patient was 'T2b'. As the patient had developed extracutaneous bone marrow involvement of lymphoma final staging at the time of diagnosis of relapse was IVB.

Secondary cutaneous lymphomas

Frequency and gender distribution

Among 54 patients whom were included in the study, 5 patients (9.25%) (2 males, 3 females; M: F ratio 0.66) were diagnosed to have secondary cutaneous lymphomas. Four (80%) were of T-cell origin and one (20%) was of B-cell origin [**Figure 8**].

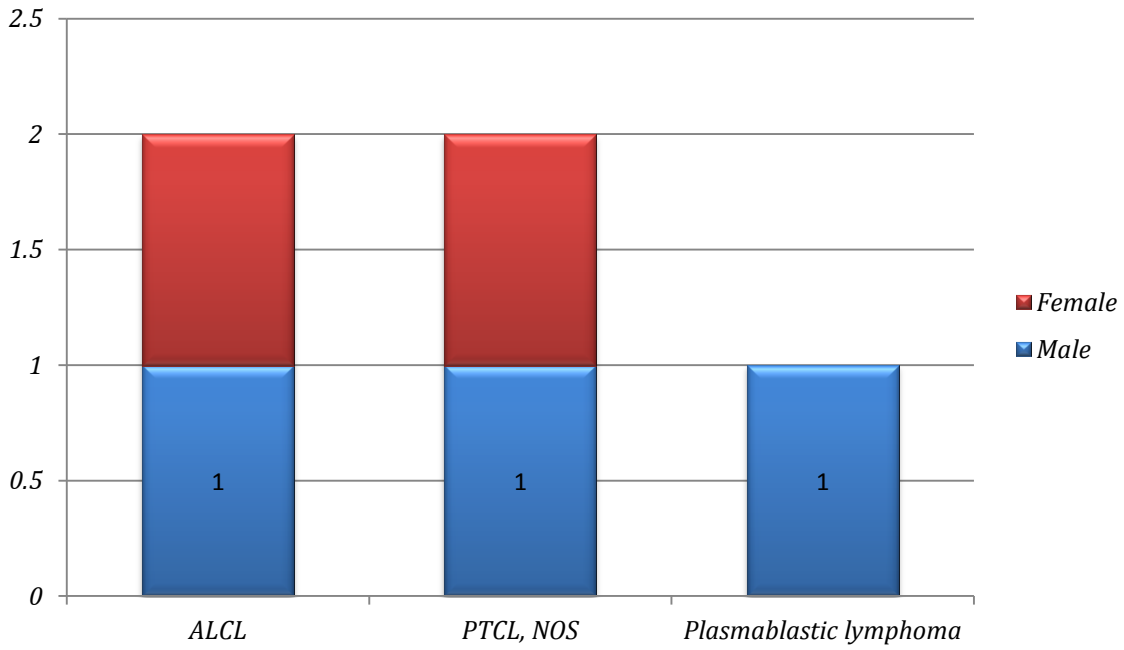


Figure 8. Various subtypes of secondary cutaneous lymphomas

Age distribution

All patients with secondary CL were adults (>16 years) [**Table 22**]

Age at diagnosis [Table 22]

Mean age at diagnosis of secondary cutaneous lymphomas was 45.2 ± 12.5 years (range, 32-59 years, median-46 years) while the mean age of diagnosis of the extracutaneous involvement was 44.6 ± 12.05 years (range, 32-58 years).

Duration of skin lesions

In 3 patients (60%) diagnosis of cutaneous and systemic involvement were made concurrently while in 1 patient (20%) cutaneous involvement was found after 6 months of systemic diagnosis and in another patient cutaneous involvement was found within 6 months of systemic diagnosis.

Types of skin lesions [Table 22]

Most common type of skin lesion noted among patients with secondary cutaneous lymphomas was nodules (4, 80%) followed by plaques (3, 60%). In two patients (28.6%) plaques coexisted with nodular skin lesions. One patient (16.7%) presented with bullous and ulcerative skin lesions.

Anatomical distribution of skin lesions [Table 22]

Common area of involvement of skin lesions in decreasing order of frequency was trunk (5, 100%) followed by lower limbs (3, 60%), upper limbs (2, 40%) and head and neck (2, 50%). 3/5 (60%) patients had more than one anatomical area of involvement. One patient had body surface area involvement of more than 50% and another patient had 11-30% of body surface area involvement and the rest of the patients (3, 60%) had involvement less than 10% of body surface area.

Systemic symptoms [Table 22]

4/5 patients (80%) presented with systemic B-symptoms including fever, weight loss, cough and pedal edema. Four patients (80%) had peripheral lymph node enlargement at the time of presentation and 3 patients (60%) had hepatosplenomegaly on clinical examination. One patient (20%) presented with ascites.

Table 22. Demographic and clinical profile of secondary cutaneous lymphomas

No	Diagnosis	AD	Gender	Skin lesion	Anatomical area	B-symptoms	T-stage
1	**ALCL**	58	F	P, NU	H, T	Absent	T3b
2	**ALCL**	45	M	N	T	Present	T3a
3	**PTCL, NOS**	55	F	P, N	T, UL, LL	Present	T3b
4	**PTCL, NOS**	32	M	P, B, U	H, T, UL, LL	Present	T3b
5	**Plasmablastic lymphoma**	33	F	N	T	Present	T3a

F – Female; M- male; P- Plaque; N –nodule; B- Bullous; NU-Noduloulcerative; U-Ulcer; H- Head and neck; T- Trunk; UL- Upper limbs; LL- Lower limbs; 'T' staging based on ISCL/EORTC proposal on TNM classification of cutaneous lymphoma other than MF/SS; AD – Age at diagnosis;

Laboratory investigations

Anaemia was noted in 3 patients (60%) but none had thrombocytopenia or leukopenia at the time of presentation. One patient (20%) had hypoalbuminemia. LDH level was elevated in 4 patients in whom the test was done. Two patients (50%) had LDH level more than 1000 IU/L. One patient was detected to have human immunodeficiency virus (HIV) infection. Viral markers in other patients were negative.

Histopathological and immunohistochemical features [**Table 23**&**Table 24**]

Epidermotropism was noted in 1 patient (20%) of secondary PTCL, NOS. Atypical lymphoid infiltrates extended into the subcutis in 2 patients (40%), deep dermis in 2 patients (40%) and superficial dermis in 1 patient (20%). Increased mitotic

activity was noted in 3 patients (60%) while angioinvasion was noted in 1 patient (20%).

Histopathological and immunohistochemical features of secondary cutaneous T-cell and B-cell lymphoma were summarised in [**Table 23** & **Table 24**].

Table 23. Histological features in various subtypes of secondary cutaneous lymphoma

	Diagnosis	Stage	Epidermot-ropism	Depth of infiltrates	Angioinva-sion
Patient 1	**ALCL**	T3b	Absent	Subcutis	Absent
Patient 2	**ALCL**	T3a	Absent	Subcutis	Absent
Patient 3	**PTCL, NOS**	T3b	Present	Deep dermis	Absent
Patient 4	**PTCL, NOS**	T3b	Absent	Superficial dermis	Present
Patient 5	**Plasmablastic lymphoma**	T3a	Absent	Deep dermis	~

Bone marrow and lymph node biopsy

Bone marrow involvement was found in 4 patients (80%) and lymph node involvement was found in 1 (20%) patient.

Table 24. Immunohistochemistry pattern in various subtypes of secondary cutaneous lymphoma

	Diagnosis	Stage	CD3	CD20	CD30	CD4	CD8	Other markers
Patient 1	**ALCL**	T3b	CD3+	CD20-	CD30+	CD4+	CD8-	CD43- ALK -
Patient 2	**ALCL**	T3a	CD3-	CD20-	CD30+			CD43+ CD79a- ALK -
Patient 3	**PTCL, NOS**	T3b	CD3+	CD20-	CD30+	CD4+	CD8-	
Patient 4	**PTCL, NOS**	T3b	CD3+	CD20-	CD30-	CD4+	CD8-	CD7+ CD56-
Patient 5	**Plasmablastic lymphoma**	T3a	CD3-	CD20-	~	~	~	CD138+ EBV+

Imaging studies

Ultrasonography

Ultrasound imaging of abdomen and pelvis was done in all five patients of whom two patients showed disease specific abnormality.

CT scan

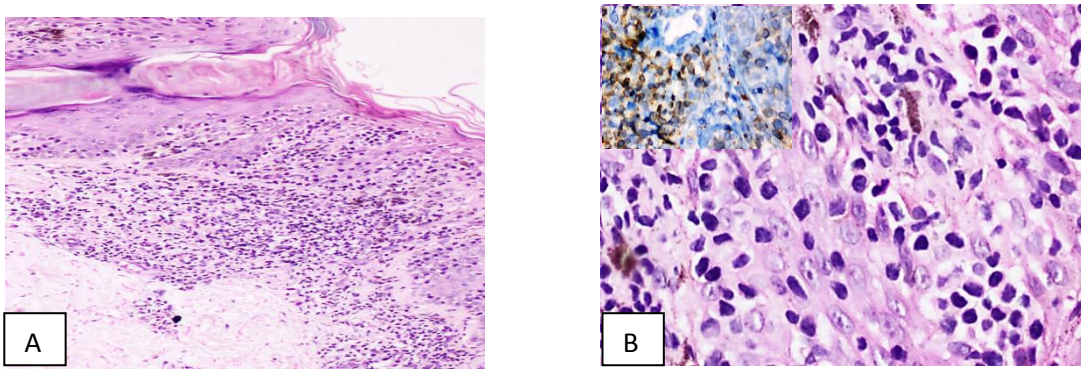
CT scan of thorax was done in 2 patients and both of them showed disease specific abnormality.

PET scan

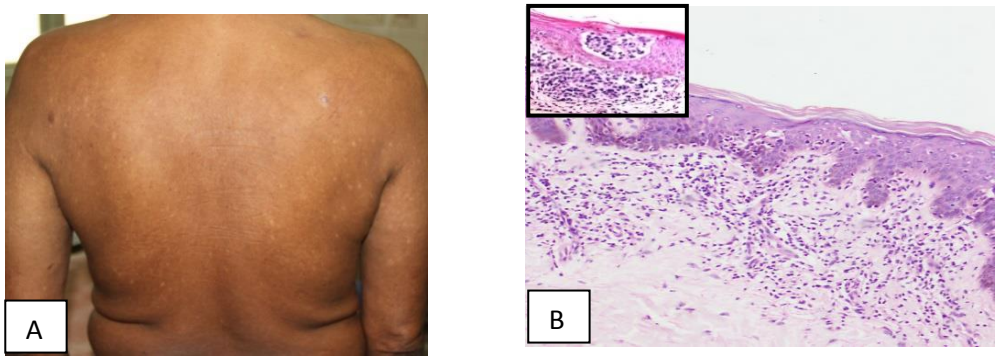
Whole body PET scan was done in patient showed features of systemic involvement of lymphoma.

Staging of secondary cutaneous lymphomas [Table 22]

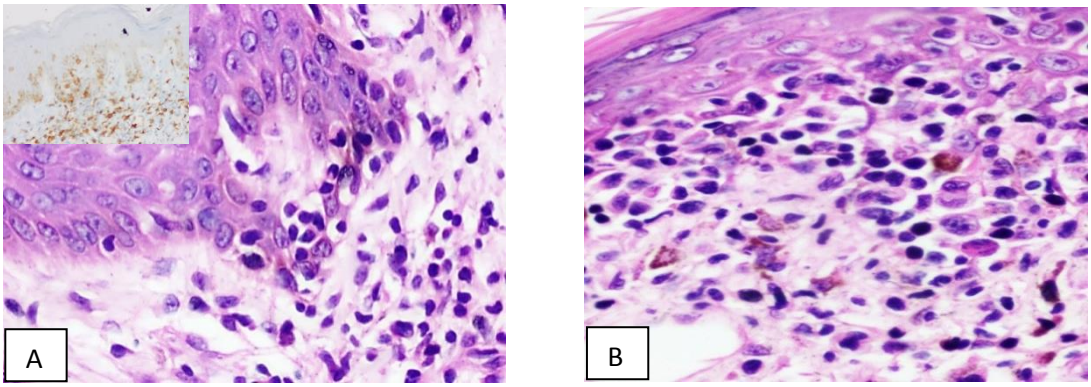
Three patients (60%) belonged to stage T3b and 2 patients (40%) belonged to stage T3a. Based on TNM classification all patients belonged to stage IVB.



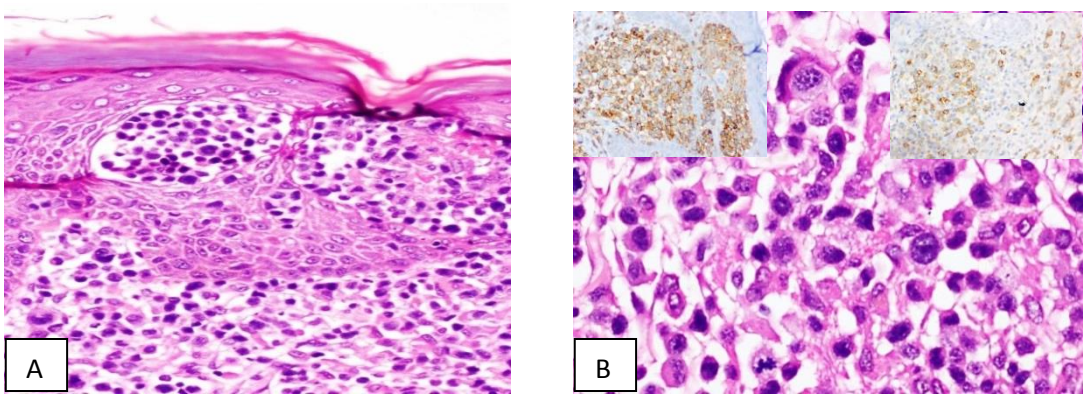
A. Lymphocytes tagging along dermoepidermal junction in early MF [H&E 100x]. B. Atypical small to medium sized cells with features of inconspicuous nucleoli and scant cytoplasm in early MF [H&E 400x]. CD3 positivity is noted on top left [inset]



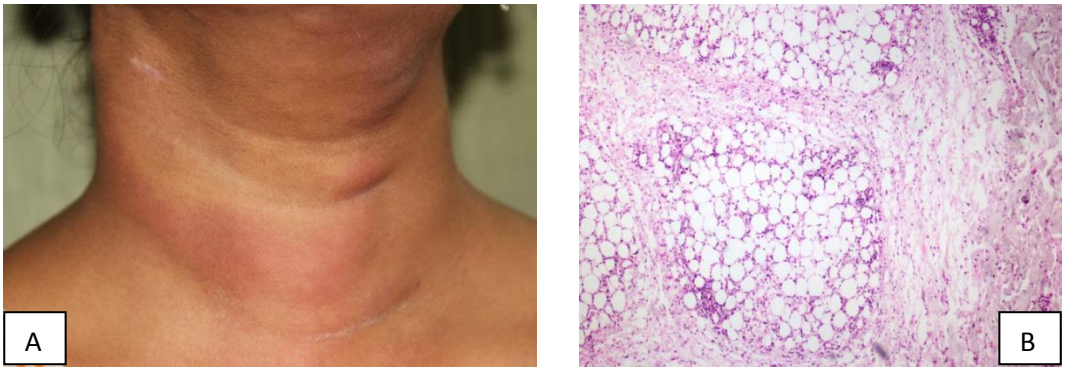
A. Hypopigmented MF; B Shows atypical lymphoid infiltrate showing epidermotropism [Inset, top right picture shows Pautrier's microabscess] H&E 100x



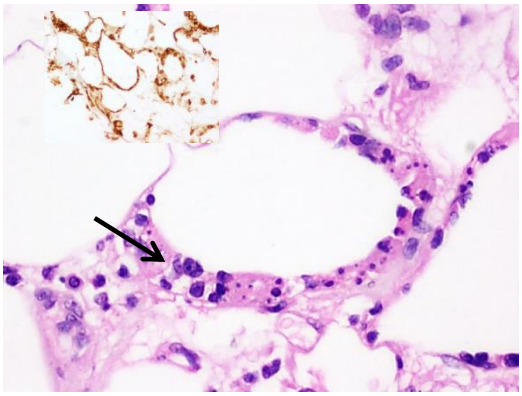
A. Hypopigmented MF: Atypical infiltrate showing epidermotropism [H&E 400x]. CD8 positivity of tumor cells [inset picture in top left]; B. Poikilodermic Mycosis Fungoides: Shows infiltrate consists of small to medium sized atypical cells with hyperchromatic nuclei, inconspicuous nucleoli and scant cytoplasm[H&E 400x]. Inset picture shows the tumor cells are positve for CD4 [top left]



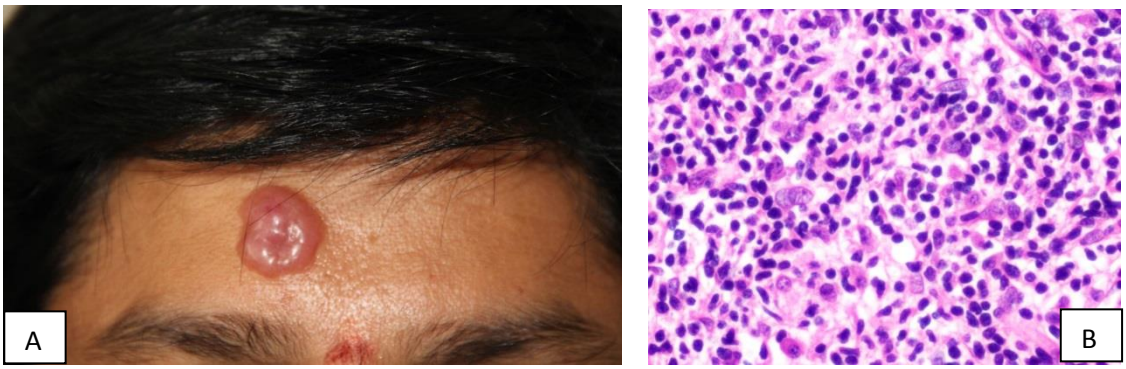
A. Mycosis Fungoides in Transformation: Shows atypical lymphoid infiltrates mixed with other inflammatory cells.H&E [100x]; B. The atypical lymphoid cells with hyperchromatic to coarse chromatin, inconspicuous nucleoli and scant to moderate amount of cytoplasm [H&E 400x]. CD4 positivity of atypical infiltrates [inset-top left] and CD30 large cells [top right]



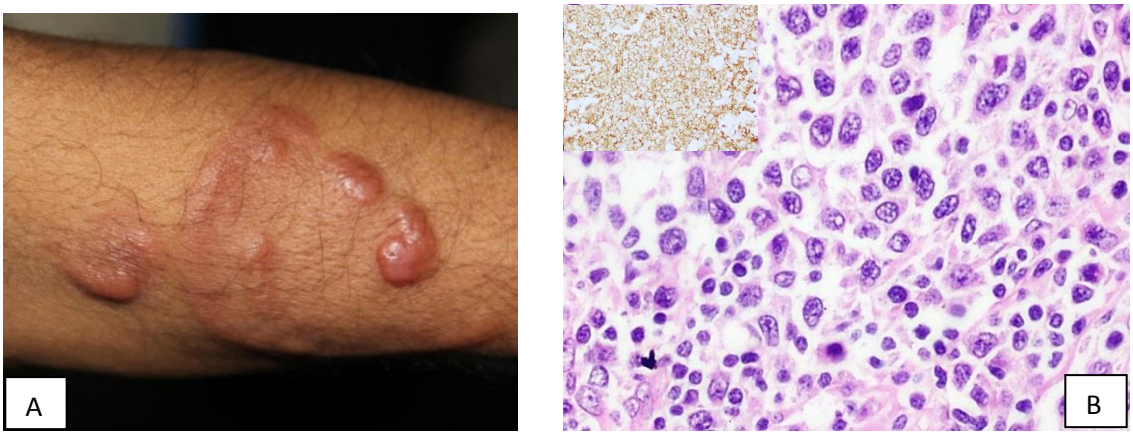
A. Subcutaneous panniculitis like T-cell lymphoma - Erythematous nodules on anterior aspect of neck; B. Subcutaenous panniculitis like T cell lymphoma: Shows diffuse infiltrates involving predominantly lobules of subcutis [H&E 40x]



SPTL - Shows rimming of adipocytes with few histiocytes engulfing nuclear dust forming bean bag cells[arrow][H&E 400x]. CD8 positivity of atypical infiltrates [inset]

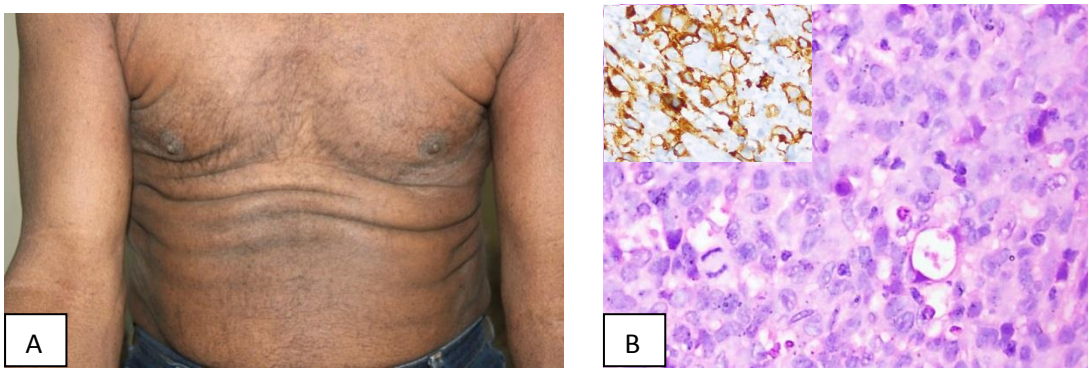


A. Tumor on forehead consistent with peripheral T-cell lymphoma, not otherwise specified; B. PTCL, NOS - Shows atypical lymphoid cells with hyperchromatic nuclei and scant cytoplasm with few admixed large histiocytes [H&E 400x]



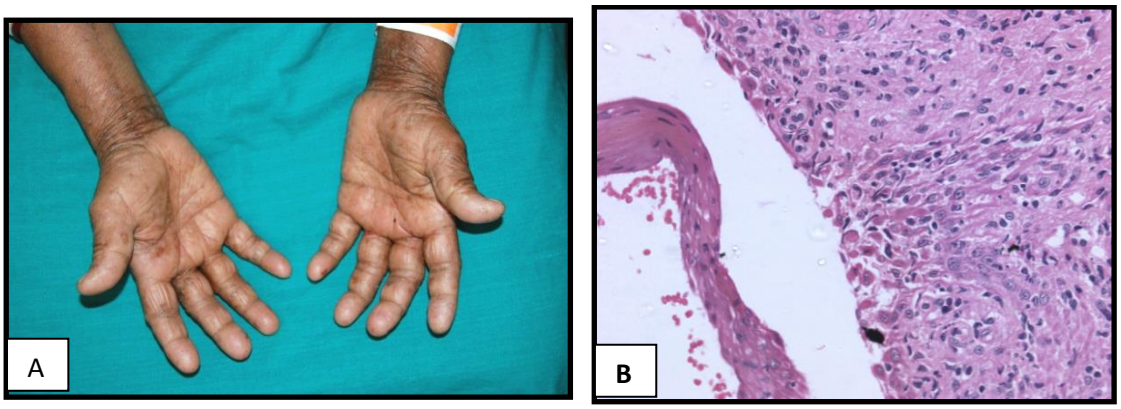
A. Erythematous plaque with annular morphology on forearm consistent with diffuse large B-cell lymphoma; B. Primary cutaneous diffuse large B cell lymphoma, other: Shows medium to large sized cells with centrocytes, few centroblast and few intermixed mature lymphocytes [H&E 400x]. CD20 positivity of tumor cells [Inset in top left]

Crusted papulovesicular lesions on face consistent with hydroa vacciniforme like T-cell lymphoma



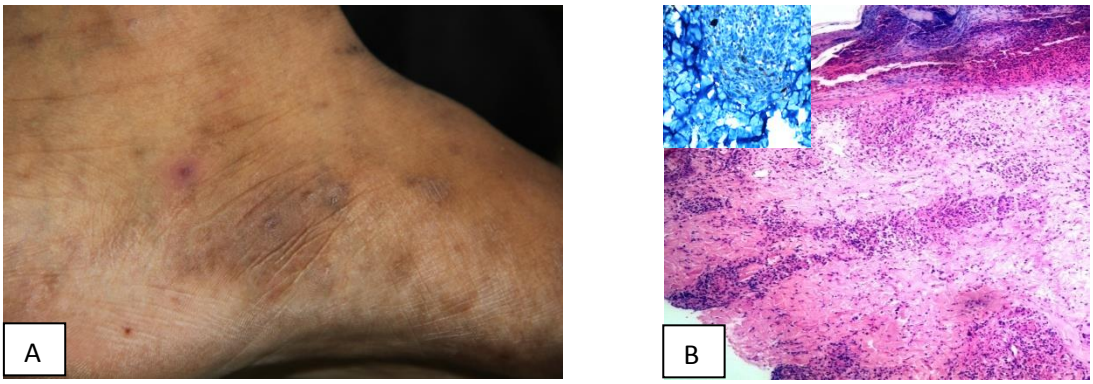
A. Chronic erythroderma consistent with anaplastic large cell lymphoma; B. Primary cutaneous anaplastic large cell lymphoma: Sheets of large cells with moderate to marked pleomorphism including mitotic figures and few large pleomorphic cells [H&E 400x]. CD30 positivity of large cells [Inset on top left]

Mycosis fungoides – Plaque stage



A. Vesicular lesions on both palms – consistent with MF; B- Cell poor intraepidermal bulla with focal epidermotropism

Coalescing papular lesions on both forearms consistent with diagnosis of LyP



A. Lymphomatoid papulosis type D – Crusted papular lesions on medial aspect of left foot;
B. Atypical lymphoid cells in epidermis [epidermotropism] Inset picture on top left corner -CD30 immunostaining of atypical lymphoid cells [x400]

DISCUSSION

Cutaneous lymphomas accounts for 19% cases of all extranodal lymphomas, second most common form of extranodal lymphoma after GI lymphomas (27%) (6). There is a paucity of data on cutaneous lymphoma from the Indian subcontinent (22,23,25). All published studies on CL are hospital based, so the true prevalence of CL in India is not known. Among CL the reported prevalence of primary CTCL was 70 - 80% and CBCL was 20-30% in Europe and West (6,7,18). In Asians the reported prevalence of primary CTCL was 75-85% while that of primary CBCL was 12-20% (8,9). In our study we found a higher prevalence of CTCL (n=48, 97.9%) while that of CBCL was 2.08% (n=1). Among the other CL, the prevalence of CD30 positive LPD and SPTL were found to be higher than that reported from the West(6,7,18). The comparative profile is shown in **Table 25.**

Table 25. Frequency of subtypes of cutaneous lymphomas in different multicentre studies around the world

Study Group	DACLG	SEER	JSCS	KOREA	DDG	Present study
Country	NL+A	US	JAPAN	KOREA	GER	IND
Year	2005	2009	2014	2014	2007	2014
Total number of patients	1905	3884	1733	517	998	49
Mature T-cell and NK-cell neoplasms	77 (1496)	71.3 (2796)	85.7 (1485)	74.2 (311)	85 (848)	97.9 (48)
MF	47	38.3	43.3	21.3	61	55.1
Primary cutaneous CD30+LPD	NA	10.2	12.0	NA	NA	22.4
Anaplastic large-cell lymphoma (ALCL)	8	NA	7.8	6	8	6.1
Lymphomatoid papulosis (LyP)	12	NA	3.8	6.2	4.4	16.3
Subcutaneous panniculitis-like T-cell lymphoma (SPTL)	1	0.6	2.0	6	<1	12.2
Peripheral T-cell lymphoma, Unspecified (PTCL)	2	20.8	5.8	9.9	~	6.1
Mature B-cell neoplasms	22.5	28.5	12.9	17.7	15	2.08
Diffuse large B-cell lymphoma, other (PCLBCL, other)	0.2	8.8	NA	1.6	~	2.04

CL - Cutaneous lymphoma; DACLG - Dutch and Austrian Cutaneous Lymphoma Group; EORTC - European Organization for Research and Treatment of Cancer; MF - mycosis fungoides; SS - Sezary syndrome; PCL - primary cutaneous lymphoma; PTL - peripheral T-cell lymphoma; SEER - Surveillance, Epidemiology, and End Results; WHO - World Health Organization; PCL - Primary cutaneous lymphoma; MF - mycosis fungoides; SS - Sezary syndrome; NL+A - Netherlands and Austria; US - United states of America; Ger - Germany

It is noteworthy that the frequency of SPTL in our study was much higher than in Europe, US, Japan and few single Institutional studies around the world (6,8,9,18,92). Among the CD30+ LPD, LyP was the common subtype. The prevalence of LyP in Germany and Asian countries varied from 3.5 - 6.5% which is lower than that seen in our study (16.3%). The frequency of cutaneous ALCL in our study (6.1%) was similar to the multicentre studies from U.S, Japan, Korea and Germany (7–9,18) [**Table 25].** The frequency of PTCL, NOS was almost similar as in Japan but much lower than those observed from Korea and U.S (6,8,9). There was one case of HVLL in our series which has not been reported from India previously. HVLL is mainly reported from Korea, China and central and South American countries (63–65). Hospital based studies from India has shown the prevalence of MF to be 36-40% which is relatively lower than that observed in our study (56.25%) (23,25) [**Table 26**]. The frequency of SPTL and PTCL, NOS were similar to an Indian study conducted in a similar setting (23). Except a study from Taiwan which showed an increased prevalence of PTCL, NOS of 16.1%, most studies including ours have shown a low prevalence of PTCL, NOS (92–95).

Table 26. Comparison of demographic profile of cutaneous lymphomas with various studies done in India

	George et al, 1999*(23)			**Doshi et al, 2011**(22)			**Burad et al, 2014**#(25)	**Present study**		
Type of study	R study			R study			R study	P study		
Duration of study	10 years			5 years			2 years	22 months		
	F	AR	M/F	F	AR	M/F	F	F	AR	M/F
MF/SS	39.4	36-70	5.5:1	74.4	20-39	3:2	36	56.25	6-74	1.25
PCALCL	3.03	~	1:0	2.12	~	2:1	8.2	6.25	41-70	2.0
LYP	~	~	~	17	41-60	2:1	8.2	16.7	2-44	1.67
SPTL	9.09	~	~	~	~	~	24.5	12.5	19-60	0.2
PTL	9.09	~	~	~	~	~	19.6	6.25	40-77	~
HVLL	~	~	~	~	~	~	~	2.08	~	1:0
CBCL	21.1	35-70	5:1	5.67	41-60	1.6:1	~	2.08	~	1:0

* Based on R.E.A.L classification; #Study included all peripheral T cell lymphomas; R-Retrospective study; P- Prospective study

Gender distribution

Like many other studies around the world including Indian studies our study also showed male preponderance among primary and secondary cutaneous lymphomas with the exception of SPTL which is more common in females(6,8,9,23,92) [**Table 27**]. The reported M/F ratio in CTCL varies from 1.02 to 1.7 and that of CBCL varies from 1.6 to 2.08 (6,92,93) **[Table 28].**

In our study the M/F ratio of CTCL was 1.28 and that of CBCL was 1:0.

Table 27. Comparison of gender distribution and mean age at diagnosis of cutaneous lymphomas across different countries

Study group	DACLG (7)		SEER (6)		JSCS (8)		KOREA (9)		Present study	
	NL+A		US		Japan		S.Korea		India	
	M/F	AD	M/F	AD	M/F	AD	M/F	AD	M/F	AD
MF	1.8	57.5	1.66	~	1.41	62	1.1	~	1.25	43
PCALCL	2.5	~	1.73	~	1.62	67.5	1.3	~	2	40
LyP	1.5	45	~	~	0.83	53.5	~	~	1.67	29
SPTL	1	~	~	~	0.55	55	0.5	~	0.2	27.5
PTL	~	~	1.82	~	1.09	68	1.1	~	~	52
HVLL	~	~	~	~	~	~	~	~	1:0	14
PCLBCL, other	~	~	1.70	~	0.90	77	1.9	~	1:0	65

M/F – Male: Female ratio; AD – Age at diagnosis

Age distribution

Results from our study show that the primary cutaneous T-cell lymphomas present earlier as compared to patients from other studies (7,8,92,93). Studies from U.S, Korea, Japan and Switzerland had demonstrated that majority of the MF present in the 6^{th} to 7^{th} decade while in our study median age at diagnosis of MF was 43 years (6,8,92,93) **[Table 27&Table 28].**

This was also seen in patients with SPTL where the disease was detected around 6^{th} decade of life (median age at diagnosis was 55 years) in a study from Japan while in our study the most common age group affected was 20-30 years of life (median age at diagnosis was 27.5 years) (8).

Table 28. Comparison of demographic profile of cutaneous lymphomas with various Institutions across the world

Country	Switzerland (92)		France (95)	Korea (94)		Taiwan (93)		Present study	
Year	2011		2006	2012		2010		2014	
Type of study	R study		P study	R study		R study		P study	
Duration	20 years		7 years	16 years		16 years		22 months	
Total patients	263		203	96		31		54	
	F (%)	M/F	F (%)	F (%)	M/F	F (%)	M/F	F (%)	M/F
CTCL	72	1.3:1	75.9	84.3	1.02:1	74	1.09:1	97.9	1.28
MF	43	1.5:1	43.3	21.9	~	13	1:1	56.25	1.25
PCALCL	8.4	1:1.2	3.5	13.5	~	8	2:1	6.25	2
LyP	5	1.4:1	7.4	9.4	~	6.5	1:1	16.7	1.67
SPTL	~	~	<1	10.4	~	3	0:1	12.5	0.2
PTL	2	~	0.5	3.1	~	16	1:4	6.3	3:0
HVLL	~	~	~	~	~	~	~	2.04	1:0
CBCL	28	1.9:1	24.1	~	~	13.5	1.6:1	2.08	1:0
PCLBCL	4	1.5:1	0	1	~	6	2:0	2.08	1:0

MF-Mycosis fungoides; PCALCL-Primary cutaneous anaplastic large cell lymphoma; LyP – Lymphomatoid papulosis; SPTL- subcutaneous panniculitis like T cell lymphoma; PTL- Peripheral T cell lymphoma unspecified; F- Frequency in percentage; M/F – Male:Female ratio; R – Retrospective study; P – Prospective study

Cutaneous lymphomas in children [Table 29]

Cutaneous lymphomas are uncommon in children and accounts for 6% of all malignant neoplasms in children (<20 years) (96). MF was the commonest among CTCL in Europe while LyP was the commonest among CTCL in Korea (26,27). Though we considered the paediatric age group in this study as age ≤ 16 years (as per Institutional norms), to facilitate the age adjusted comparison between published studies we considered the patients who have been diagnosed as cutaneous lymphoma before the age of 20 years. In our study, MF was the most common CL which occurred in 4/9 patients (age ≤ 20 years). The frequency of MF was similar to the observation made in Europe (27) [**Table 29**]

SPTL and lymphomatoid papulosis occurred in 2 patients each (n=2, 22.2%). Median age of diagnosis of MF and LyP in our study (13 and 9.5 years) was similar to the Korean study (13.8 and 8 years) but slightly lower than the European study (17 and 14 years) (26,27) [**Table 29**].

Table 29. Demographic profile of paediatric cutaneous lymphomas compared with studies across the world

CTCL subtypes	West (n=69)				Korea (n=41)				Present study (n=9)			
	R study – 1960-2002				R study – 1990 -2012				P study – 22 months			
	Age cut off ≤ 20 years				Age cut off ≤ 20 years				Age cut off ≤ 20 years			
	%	M/F	AR	Med	%	M/F	AR	Med	%	M/F	AR	Med
MF	34.7	1:1	3-20	17	27.6	5:4	7-19	13.8	44.4	3:1	5-14	13
LyP	17.7	5:6	3-19	14	34.5	7:3	0.4-17	8	22.2	0:2	2-17	9.5
SPTL	1.6	0:1	~	20	3.4	1:0	~	7	22.2	0:2	~	19
HVLL	~	~	~	~	~	~	~	~	11.1	1:0	~	14

M: F Male:Female ratio; R- Retrospective study; P- Prospective study; AR – Age range; Med – Median age at diagnosis

Mycosis fungoides (MF)

The median duration of disease prior to diagnosis was about 6 years in our study and this was similar to data from other studies where it was found to be 4-6 years (7,36,38,92). A previous Indian study had showed a median duration of 96 months which was slightly more than the current observation (23). This might be due to increased awareness among the population regarding skin diseases. Various types of MF have been described like poikilodermatous, hypopigmented, ichthyosiform, palmaris et plantaris, bullous/vesicular, pustular, pigmented purpura like and hyperkeratotic verrucous like MF in the literature (16,97,98). In our study the variants seen included poikilodermatous variant, hypopigmented variant and vesicular variant of MF were seen. One patient in our study presented with dyshidrotic vesicles on palms and soles consistent with the diagnosis of MF, a rare atypical variant reported in literature (99,100). Incidence of poikilodermatous MF in a study from U.K was reported to be 11% while in our study the poikiloderma was seen in 25.9% (98). Hypopigmented MF had been increasingly reported in Asians and Afro- Americans and rarely in light skinned people with good response to therapy (97,101,102). Hypopigmented skin lesions were reported more commonly in paediatric age group than adults (72.4% vs. 1%) (103). In our study 5/27 (18.5%) patients presented with hypopigmented skin lesions of which one was a child (3.7%). Involvement of palms and soles in the course of MF was reported to be 11.5% while in our study 14.8% (4/27) patients had palmoplantar involvement which was confirmed with biopsy in 2 patients (104).

Large population studies in MF had shown that most of the MF patients present in early stage (I-IIA) (70-75%) than advanced stage (IIB-IVB) (20-25%) of the disease

(40,105). In our study 81.5% (n=22) patients presented in early stage (I-IIA) and 18.5% (n=5) patients presented in advanced stage (IIB-IVB).

The reported occurrence of extra cutaneous involvement in MF is 6-7% which correlates with type and extent of skin lesions (36,38). In our study extra cutaneous involvement was noted in 4/27 patients (14.8%). The reported occurrence of bone marrow involvement in MF/SS is 5.6% and is usually seen in advanced stages of disease (stage T4) (105). In our study 3% had bone marrow involvement (1 each in stage T2b, T3 and T4). Biopsy proven lymph node involvement was seen in 3.7% of our patients, while the prevalence of the same in MF was reported to be around 3-7% (36,38,40,105). It have been reported that 7% patients have Sezary cells in the peripheral smear while in our study we had 1 patient (3.7%) with Sezary cells in the peripheral smear (38).

Frequency of Pautrier's microabscesses in patients of MF varied from 4-37% and the same in our study was seen in 33% of patients (n=9) which was in conjunction with other studies (35). Large cell transformation (>25% of cells) may occur in 8-55% patients of MF and reported to be associated with poor prognosis (106). In our study the same was noted in 2 patients (7.4%) and each one belonged to T3 and T4 stage respectively.

CD8 positive MF was reported to be as high as 32% in an Indian study compared to 25% in an Italian study (22,107). Prognosis of CD8+ mycosis fungoides is reportedly better than CD4+ mycosis fungoides (108). In our study CD8 mature T-cell positive phenotype was seen in 3/27 (16.7%) patients. All three patients presented with hypopigmented macules and belonged to T2a stage.

TCR gamma gene rearrangement study

Gold standard for diagnosing MF is histopathological examination of a skin biopsy. Loss of T-cell differentiation antigens (CD2, CD3, CD5 and CD7) diagnosed using immunohistochemical studies and detection of clonal T-cell population in skin biopsies using PCR aids an additional value in diagnosis of MF (86). Criteria proposed by International Society for Cutaneous lymphomas (ISCL) for diagnosis of early MF (32) was not implemented in this study due to lack of facilities for quantitative analysis of immunohistochemistry markers in our Institution. Clonality detection in CTCLs varies from 40-90% and is dependent on the PCR protocol used for the detection of clonal cells (11,12,14,15,87,109). Three studies which used Genescan for detection of clonality had showed a detection rate of 66-73% in early stage MF. In our study the detection rate was slightly lower (61.5%) (14,15,110) [**Table 30**]. As 'T' score of the disease advanced clonality detection rate proportionately increased (12). Ponti et al demonstrated a clonality percentage of 83.5% in all CTCLs and 73.75% in T1/T2 stages of MF while in our study the clonality detection percentage was 61.5% in T1/T2 stage of MF (12).

Table 30. Clonality detection rate in early stage MF (I/IIA) among different studies using similar PCR protocol

Study	Country	Published year	Clonality detection rate
Klemke et al(14)	Germany	2002	8/12 (66.7%)
Sandberg et al(15)	Netherlands	2003	5/8 (63%)
Goeldel et al(110)	France	2010	27/37 (73%)
Present study	India	2014	8/13 (61.5%)

Only very few studies are available till date that have studied the association between histopathological features and clonality detection (12,87). Tok et al had demonstrated clonal TCR gamma gene rearrangements in 73% of the specimens' non diagnostic for CTCL, 71% of those suggestive of CTCL, and 74% of those diagnostic of CTCL (87). He suggested that histology and molecular biology do not necessarily correlate which was demonstrated by the clonal persistence in 30–40% of patients with CTCL displaying complete clinical and histological response. When we compared the clonality detection with histological grading of early MF (stage T1/T2) proposed by Guitart et al, we found that the clonality detection was 50% (3/6) in patients where the diagnosis of MF could not be excluded by histopathological grading whereas in a study done by Ponti et al the clonality detection was 40.6% (13/32) in the same group of patients (12) **[Table 31].**

In the same study the clonality detection was 87.6% (64/73) in patients suggestive of MF whereas in our study it was found to be 80 % (4/5) **[Table 31]**.

Loss of lineage of T-cell differentiation antigens was found in 8/13 (61.5%) of which 6/8 (75%) patients had showed monoclonal pattern and 2/5 (40%) patients had showed polyclonal pattern. Ponti et al demonstrated higher percentage (93.9%) of loss of lineage of T-cell differentiation antigens while our study showed 75% of the same (12).

Though the statistical significance of clonality detection with histopathological grading of MF could not be demonstrated it was evident that in patients with histopathological diagnosis suggestive of MF had increased clonal detection rate. We might need a large population studies on the same prior to any conclusion. False negativity in detection of clonality could be attributed to low density of infiltrated

tumour cells in biopsy specimens of early MF, partial or incomplete rearrangement of the TCR, somatic hypermutation or TCR region translocation (86,90). However the patients of both groups (polyclonal and monoclonal) will be followed up in the future for the course of disease progression.

Table 31. Comparison of clonality detection rate based on histology category among different studies

Histological and immunohistochemical diagnosis of MF	Present study	Ponti et al (12)	Tok et al*(87)
MF cannot be excluded	50% (4/8)	40.6% (13/32)	73% (8/11)
Probable/suggestive of MF	80% (4/5)	87.6% (64/73)	71% (6.6/9.3)

* Study included all CTCL specimens and TCR gene rearrangement assay was done using PCR/DGGE

Primary cutaneous ALCL

Two patients (66.7%) in our study presented with nodular skin lesions which is the most common morphological skin lesions in most other studies (45,111). The other patient in our study presented with erythroderma. Only a few cases of erythrodermic anaplastic large cell lymphoma has been reported in literature (48,112). Male predominance noted in our study correlated with observation by various authors (111,113). While extra cutaneous dissemination can occur is 10% of patients , none of the patients in our study had extra cutaneous involvement (111). All three patients in our study showed ALK negativity which was consistent with the diagnosis of primary cutaneous ALCL as reported in many studies (49,114).

Lymphomatoid Papulosis (LyP)

Male predominance is noted in LyP studies around the world as in our study (M: F, 1.67). Most common histologic variant of LyP was type A (79%) followed by type C (6.7%) and type B (5.08%) (45). In our study the most common variant of LyP was type A (n=5, 62.5%) followed by type B (n=2, 25%). One patient had a newly described rare type D (1, 12.5%) variant. In a large study comprising of 219 patients with primary and secondary CD30+ LPD, 19% (23/118) patients had associated malignant lymphoma either before, after or concurrent with LyP and median follow up of the study was 77 months (45). In our study none of the patients had any evidence of concurrent lymphoma at the time of presentation. Longest duration of disease prior to diagnosis in our study was 204 months and median duration was 12 months.

Subcutaneous panniculitis like T-cell lymphoma (SPTL)

SPTL was included as a distinct entity in WHO-EORTC 2005 classification and it is defined as cytotoxic T-cell lymphoma characterized by subcutaneous infiltrates of small medium or large pleomorphic T-cells with α/β T-cell phenotype. Our study showed a frequency of 12.2% (n=6) of all primary CTCLs. M/F ratio of SPTL reported in literature was 0.5 while in our study the M/F ratio was 0.2 (56). The median age at diagnosis of SPTL reported in literature was 36 years (range 9-79 years) (56) while in our study the median age of diagnosis was 27.5 years. The morphological presentation of SPTL was nodules/plaques (100%) and majority of the patients has multifocal involvement (78%). Extremities are more commonly involved than trunk and face (56). In our study all patients presented with nodules and multifocal involvement was noted in 66.7% of patients. Trunk was the most common area of involvement in our study. CD56 positivity, hemophagocytic syndrome, angioinvasion, skin ulcers, liver

dysfunction and elevated LDH are reported to be associated with an unfavourable prognosis. (56–58). Incidence of abnormal laboratory values (anaemia, leucopenia, thrombocytopenia, elevated liver function test) have been reported to be 29% and B-symptoms are reported to occur in 59% of patients (56). In a study comprising 22 cases of SPTL incidence of elevated liver enzymes and elevated LDH were reported to be 59% and 64% respectively(58). In our study B- symptoms were noted in 66.7% patients while elevated LDH was noted in 5 patients (83.3%) and abnormal liver transaminases were noted in 2 patients (40%).

Haemophagocytosis was observed in 15% of patients while in our study the same was noted in one patient (1/6) (16.6%) which was similar to the observation by Willemze et al (56). Though adipocyte rimming by neoplastic T-cells are not specific or diagnostic of SPTL it is commonly observed in many patients of SPTL (56,57). This feature was also reported in patients of tumour stage MF, aggressive epidermotropic CD8+ T-cell lymphoma, extranodal NKTL and few secondary cutaneous B-cell lymphomas (115).

Peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS)

Patients who do not fit into any of the well-defined entities of CTCL are classified under PTCL, NOS (7). Most common morphological lesions reported in literature under this entity were generalized nodules or tumours while in our study all three patients (100%) presented with nodular skin lesions of which two patients had multifocal involvement (BSA>30%). There is no site predilection reported for this category of CTCL (67). Prognosis of this entity is poor irrespective of the type and extent of skin lesions with 5 year survival rate of less than 20% (7,24,67). Duration of skin lesions prior to diagnosis varied from 1 to 100 months (median, 6 months) while in

our study median duration of skin lesions prior to diagnosis ranged from 3 to 6 months (median, 5 months) (67). CD30 staining & CD56 expression are usually negative which was the finding in our study also (67).

Hydroa vacciniforme like T-cell lymphoma

Hydroa vacciniforme like T-cell lymphoma, an EBV+ T-cell lymphoproliferative disorder of childhood (updated in 2008 WHO classification) had been reported increasingly (2-7%) from Central America, South America and Far East countries (8,9,61). Our patient presented with systemic B-symptoms as in most other patients reported in literature (65).

Median age at diagnosis reported in literature was 8 years while our patient was diagnosed at the age of 14 years (65). A large study on HVLL comprising of 20 patients showed the involvement of sun exposed areas in all patients (100%). The morphology and distribution of lesions of our patient were similar to that reported in literature. The presence of concurrent hepatosplenomegaly and lymphadenopathy seen in our patient has been reported in literature. The infiltrate was monoclonal and IHC pattern of CD3+, CD8+, granzyme B+, T1A-1+ and EBV-LMP positivity was typical of HVLL (65). EBV antibody in the serum was positive in our patient.

Secondary cutaneous lymphomas

Most common lymphoma which present with secondary cutaneous involvement is diffuse large B-cell lymphoma (6). In a large population study of cutaneous lymphoma, diffuse large B-cell lymphoma with secondary cutaneous involvement was around 27% while in our study the most common secondary cutaneous lymphoma were 2 cases of ALCL and PTCL, NOS (40%).

Anaplastic large cell lymphoma

Systemic anaplastic large cell lymphomas were distinguished by the expression of ALK antigen and negative expression of the same in systemic ALCL correlates with poor prognosis (45) although both subtypes warrant treatment with polychemotherapy. Involvement of skin in patients of systemic ALCL has been reported to be 12.5% (116).

Median age at diagnosis is 59.5 years and B-symptoms reportedly to occur in 27% patients while in our study the mean age at diagnosis was 51.5 years and one patient had B-symptoms (45). M/F ratio of secondary cutaneous ALCL was 0.83 while in our study the M/F ratio was 1:1. The reported median duration to develop skin lesions following the diagnosis of systemic ALCL was 53 months while in our patient the same was 6.5 months (45).

Peripheral T-cell lymphoma, not otherwise specified

Secondary or concurrent involvement of skin in PTCL, NOS ranged from 19-55% patients as reported in a study (67). Most common skin lesions were multifocal erythematous plaques to tumours and nodules (67). As reported in earlier studies our patients also presented with multifocal polymorphic skin lesions (plaques and tumours). Mean age of diagnosis in our study was 43.5 years while in a study comprising 82 patients with PTCL, NOS it was reported to be 65 years (range 8-85 years) (67). Most common immunohistochemistry observed in the same study was CD4+/CD8- (12/17, 70.5%) while in our study both patients showed the CD4+/CD8- immunohistochemistry pattern (67).

Plasmablastic lymphoma (PBL)

Plasmablastic lymphoma, a rare B-cell non-Hodgkin lymphoma often associated with HIV infection and Epstein-Barr virus (EBV) infection (117). PBL accounts for 2.6% of all acquired immunodeficiency syndrome related malignant lymphomas (118). Male predominance is noted in PBL (77%) and median age at diagnosis was 46 years (range - 1.2 to 87 years) (117). Cutaneous involvement is noted in 12% patients with plasmablastic lymphoma. Overall survival rate was between 6 to 12 months (117). In our study a female patient with HIV infection was diagnosed to have plasmablastic lymphoma with cutaneous involvement. Both systemic and cutaneous involvement was diagnosed concurrently. Age at diagnosis of our patient was 33 years. Most common morphological cutaneous presentation of PBL reported in literature was nodules which was a finding in our patient also (117,119).

CONCLUSIONS

- CTCL was the most common subtype of CL seen in our study which was similar to data from studies reported from Asia and the West. However the proportion of CTCL was higher than that reported from other countries.
- Male preponderance was noted in all subtypes of cutaneous lymphomas except SPTL where the males were affected less frequently (M/F -1:5)
- MF was the commonest among CTCL comprising 56.25% of all CTCLs.
- Majority of MF patients belonged to stage I & IIA as has been reported from other studies.
- Duration of disease prior to disease in MF was significantly longer than other subtypes of CTCLs ($P < 0.001$).
- Unusual clinical types of MF seen included hypopigmented MF and dyshydrosiform MF. MF with palmoplantar involvement was also seen in 14.8% patients.
- Among the unusual variants of CTCL (other than MF) seen in our study were one case each of erythrodermic ALCL, LyP type D and HVLL.
- 16.7% of patients with lymphoma were in the paediatric age group (≤ 20 years). The most common type of CTCL seen was MF (44.4%).
- Monoclonality detection in early MF of 61.5% in our study was slightly lower than the detection rate in various studies across Europe using a similar protocol.
- In our study we did not find any significant correlation between the histological grades of MF proposed by Guitart et al and clonality detection. However the clonality detection rate was higher in patients with histopathological diagnosis suggestive of MF than patients in whom the diagnosis of MF could not be excluded.

- The loss of lineage of T-cell differentiation antigens was higher in early MF patients in whom monoclonality was detected; however the difference was not statistically significant.

LIMITATIONS

- The study is from a single centre and done over a limited period of time (22 months), so the number of patients studied is small.
- Clonality pattern was studied only in 13 patients of patch/plaque (Stage I/IIA) mycosis fungoides where in the histological diagnosis of mycosis fungoides could not be excluded or the diagnosis was suggestive but not confirmatory. This is because of the high cost of the procedure.

RECOMMENDATIONS

- Multicentre studies are needed to study the prevalence and clinical profile of cutaneous lymphomas seen in India.
- A cutaneous lymphoma registry should be started for the same and all the data entry should be facilitated by an electronic database for the convenience of physicians across the country.

SUMMARY

Background

Cutaneous lymphoma, an extranodal non-Hodgkin lymphoma on skin is distinct from non-cutaneous lymphomas in clinical profile, course, prognosis and treatment. So it is important to know the epidemiology of cutaneous lymphomas, frequency, gender and age distribution and distinct clinicopathological profile of each subtype. Though there are few multicentre studies across the globe, studies from India were sparse. So we decided to take up this study to find epidemiological data and clinicopathological profile of cutaneous lymphomas in a hospital based setting.

Moreover diagnosis of mycosis fungoides was always been a challenge to both dermatologist and pathologist particularly to differentiate the early stages of mycosis fungoides from benign dermatoses. In addition to clinical picture, histopathology and immunohistochemistry markers, molecular study aids as a tool in diagnosis of early stage of mycosis fungoides. In this background we decided to do look the role of TCR gene rearrangement study in early stage of mycosis fungoides to find the role of clonality studies in early stage of mycosis fungoides.

Objective

Our primary objective was to study the clinicopathological profile of cutaneous lymphomas in a hospital based setting. Secondary objective was to study the role of TCR gene rearrangement study in early stage mycosis fungoides.

Methods

A hospital based cross sectional study was conducted in department of Dermatology, Venereology and Leprosy, Christian Medical College, Vellore over period of 22 months with approval from the Institution Review Board (IRB.No: 8050). Patients with clinical features of cutaneous lymphomas were included in the study after confirmation with biopsy. Immunohistochemical markers were done in all patients to confirm the diagnosis as a part of standard operating procedures. Patients were informed prior to inclusion in the study and a written consent was obtained from the patients and guardians in patient of children. Demographic details, clinical data, laboratory values, imaging and histopathological findings were recorded in a proforma. TCR gamma gene rearrangement study was done in patch/plaque (Stage I/IIA) mycosis fungoides where the histological diagnosis of mycosis fungoides could not be excluded or the diagnosis was suggestive but not confirmatory. Detection of clonality was interpreted in correlation with clinical and histopathological features. Data on clinical and pathological features were expressed in numbers and percentages.

The role of TCR gene rearrangement in detection of monoclonality in early stage MF was calculated with 95% confidence interval. *P* value was calculated using Fisher's exact test wherever applicable.

Results

A total of 54 patients with cutaneous lymphomas were included in the study. There were a total of 30 males and 24 females (M/F-1.25). The mean age at diagnosis of cutaneous lymphoma was 39.9±18.3yrs (range 2-77years).

Primary cutaneous T-cell lymphomas were the commonest among all cutaneous lymphomas (n=48, 88.9%) followed by secondary CTCL (n=4, 7.4%), primary CBCL (n=1, 1.85%) and secondary CBCL (n=1, 1.85%). The most common type of CTCL in the paediatric age group (≤ 20 years) was MF (n=4, 44.4%) followed by LyP (n=2, 22.2%), SPTL (n=2, 22.2%) and HVLL (n=1, 11.1%).

Among subtypes and its variants, mycosis fungoides was the commonest (27, 50%) followed by CD30+ LPD (n=11, 20.3%), SPTL (n=6, 11.1%), PTCL, NOS (n=3, 5.6%), HVLL (n=1, 1.85%) and PCLBCL (n=1, 1.85%). 2 patients (4.2%) of secondary ALCL, 2 patients (4.2%) of secondary PTCL, NOS and 1 patient (1.85%) of plasmablastic lymphoma were also seen during the study period.

Mean age at diagnosis of MF, CD30+ LPD, SPTL and PTCL, NOS were 41.1±18.8 years, 33.8±17.1 years, 33.6±17.3 years and 56.3±18.9years respectively. Duration of disease prior to diagnosis of MF was much longer when compared to other subtypes of cutaneous lymphomas (median, 72 months) ($P < .001$). Patches were the commonest morphological skin lesions among MF patients while all patients with SPTL classically presented with subcutaneous nodular skin lesions and patients with LyP presented with papular skin lesions.

Unusual clinical types of MF in our study included hypopigmented MF and dyshidrosiform MF. MF with palmoplantar involvement was also seen in 14.8% patients. Among the unusual types of CTCL (other than MF) seen in our study were one case each of erythrodermic ALCL, LyP type D and HVLL.

Clonality detection rate among early stage MF in our study was 61.5%. But statistical significance of the same with histological grading and immunohistochemistry findings was not found. However we found that the clonality detection was higher in

patients in whom the histopathological diagnosis was suggestive of MF than the patients in whom the histopathological diagnosis of MF could not be excluded.

CONCLUSION

In our study of 54 patients with cutaneous lymphomas, CTCL was the most common subtype as reported in earlier studies from Asian countries and the West. However the proportion of CBCL was lower than that reported from other countries. Female preponderance was noted in SPTL and rest of the subtypes showed a male preponderance in occurrence. MF was the commonest among CTCL comprising 56.25% of all CTCLs. The unusual types of CTCL seen in our study included erythrodermic MF, hypopigmented MF, dyshidrosiform MF, LyP type D and HVLL. Duration of disease prior to disease in MF was significantly longer than other subtypes of CTCLs ($P < .001$). Monoclonality detection in early MF was slightly lower than the detection rate in various studies across Europe using a similar protocol. In our study we did not find any statistically significant correlation between the histological grades of MF, immunohistochemical studies and clonality detection in early MF. Usefulness of TCR clonal rearrangement studies in early MF has to be concluded with large population studies. A cutaneous lymphoma registry should be started and all the data entry should be facilitated by an electronic database for the convenience of physicians across the country.

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ANNEXURE I

ANNEXURE I (A):

The International Society for Cutaneous Lymphomas (ISCL)/European Organization of Research and Treatment of Cancer (EORTC) revised clinical staging and classification of mycosis fungoides and Sezary syndrome

Tumour stage (skin)

- T1: limited patches, papules, and/or plaques covering <10% of skin surface
  - T1a: patch only
  - T1b: plaque with or without patch
- T2: patches, papules, or plaques covering 10% or more of skin surface
  - T2a: patch only
  - T2b: plaque with or without patch
- T3: one or more tumours equal to or greater than 1 cm diameter
- T4: confluence of erythema, covering 80% or more of body surface area.

Nodal stage

- N0: no clinically abnormal peripheral lymph nodes; biopsy not required
- N1: clinically abnormal peripheral lymph nodes; histopathological involvement, no atypical lymphocytes
  - N1a: clone-negative
  - N1b: clone-positive
- N2: clinically abnormal peripheral lymph nodes; histopathological involvement, aggregates of atypical lymphocytes
  - N2a: clone-negative

- N2b: clone-positive

- N3: clinically abnormal peripheral lymph nodes; histopathological involvement, partial or complete effacement of nodal architecture by atypical lymphocytes or neoplastic cells; clone-positive or -negative

- N_x: clinically abnormal peripheral lymph nodes; no confirmed histological involvement.

Metastatic stage (visceral)

- M0: no visceral disease

- M1: visceral disease.

Blood stage

- B0: No haematological involvement; <5% atypical or Sezary's cells in peripheral blood

  - B0a: clone-negative

  - B0b: clone-positive

- B1: Sezary's count 5% or more of peripheral blood lymphocytes, but does not meet criteria of B2

  - B1a: clone-negative

  - B1b: clone-positive

B2: 1000/microliter or more Sezary's cells; clone-positive.

ANNEXURE I (B)

ISCL/EORTC revision to the staging of mycosis fungoides and Sezary syndrome

STAGE	T	N	M	B
IA	1	0	0	0,1
IB	2	0	0	0,1
II	1,2	1,2	0	0,1
IIB	3	0-2	0	0,1
III	4	0-2	0	0,1
IIIA	4	0-2	0	0
IIIB	4	0-2	0	1
IVA1	1-4	0-2	0	2
IVA2	1-4	3	0	0-2
IVB	1-4	0-3	1	0-2

ANNEXURE I (C)

The SS diagnostic criteria were:

(i) Erythroderma and peripheral lymphadenopathies

(ii) Peripheral blood involvement by circulating Sezary cells (SC)

(iii) Cutaneous biopsy-proven CTCL.

Peripheral blood involvement was defined, according to the criteria recently proposed by the International Society for Cutaneous Lymphoma, only in the presence of two major criteria: i) An absolute circulating SC count > 1000 mm^3 ; ii) PCR detection of a dominant TCR-γ gene rearrangement in the peripheral blood.

Adjunctive criteria were:

(i) CD4⁄CD8 ratio > 10;

(ii) Circulating CD4+CD7– ‡ 40%;

(iii) Aberrant expression of T-cell markers; and

(iv) Chromosomally abnormal T-cell clone.

ANNEXURE I (D)

Pathological staging for lymph nodes in mycosis fungoides

Updated ISCL/EORTC Classification	**Dutch system**	**NCI-VA classification**
N1	Grade 1: dermatopathic lymphadenopathy (DL)	LN0: no atypical lymphocytes LN1: occasional and isolated atypical lymphocytes (not arranged in clusters) LN2: many atypical lymphocytes or in 3-6 cell clusters
N2	Grade 2: DL; early involvement by MF (presence of cerebriform nuclei > 7.5 um)	LN3: aggregates of atypical lymphocytes; nodal architecture preserved
N3	Grade 3: partial effacement of LN architecture; many atypical cerebriform mononuclear cells (CMCs) Grade 4: complete effacement	LN4: partial/complete effacement of nodal architecture by atypical lymphocytes or frankly neoplastic cells

ANNEXURE II

Histopathological criteria for diagnosis of MF as proposed by Guitart et al

Major criteria

1) Density of infiltrate at low power

Grade 0= scant infiltrate

Grade 1= Mild perivascular superficial infiltrate

Grade 2= moderately dense perivascular or band like infiltrate without thickening of papillary dermis

Grade 3= Dense confluent infiltrate with thickening of the papillary dermis and involvement of the reticular dermis.

1) Epidermotropism at medium power

0= None

1= Local basal epidermotropism and/or single Pautrier microabscesses or a few couplets/triplets or scattered lymphocytes without spongiosis

2= Extensive basal and/or two or more Pautrier microabscesses

3= Extensive epidermotropism with more lymphocytes than keratinocytes.

2) Atypia at high power

0 = No atypia

1 = Mild atypia

2= Moderate atypia

3= uniformly atypical or pleomorphic cells, many mitotic figures, few small round reactive lymphocytes

Minor criteria

1- Reticular fibroplasias of the papillary dermis around single lymphocytes

1 or 2- Primarily intraepidermal atypical lymphocytes

1-Lymphocytic infiltrate without inflammatory features

Scoring for diagnosis

Score	Diagnostic category
0-2	Perivascular/interface dermatitis or other specific dermatoses
3-4	Atypical lymphocytic infiltrate(MF cannot be excluded)
5-6	Atypical lymphocytic infiltrate suggestive of MF
7+	Mycosis fungoides

ANNEXURE III (A)

ISCL/EORTC proposal on TNM classification of cutaneous lymphoma other than MF/SS

T

T1: Solitary skin involvement

T1a: a solitary lesion <5 cm diameter

T1b: a solitary >5 cm diameter

T2: Regional skin involvement: multiple lesions limited to 1 body region or 2 contiguous body regions

T2a: all-disease-encompassing in a <15-cm-diameter circular area

T2b: all-disease-encompassing in a >15- and <30-cm-diameter circular area

T2c: all-disease-encompassing in a >30-cm-diameter circular area

T3: Generalized skin involvement

T3a: multiple lesions involving 2 noncontiguous body regions

T3b: multiple lesions involving >3 body regions

N

N0: No clinical or pathologic lymph node involvement

N1: Involvement of 1 peripheral lymph node region that drains an area of current or prior skin involvement

N2: Involvement of 2 or more peripheral lymph node regions† or involvement of any lymph node region that does not drain an area of current or prior skin involvement

N3: Involvement of central lymph nodes

M

M0: No evidence of extracutaneous non–lymph node disease

M1: Extracutaneous non–lymph node disease present

ANNEXURE III (B)

ISCL/EORTC recommendations for staging evaluation in cutaneous lymphomas other than MF/SS

Complete history/review of systems and physical examination

Laboratory studies

Complete blood count, comprehensive serum chemistries, serum LDH

Whenever indicated, relevant flow cytometric studies of peripheral blood mononuclear cells

Imaging studies*

CT of chest, abdomen and pelvis with contrast alone or with whole-body PET (18F-FDG); include CT or ultrasound of neck if clinically indicated

Whole-body integrated PET/CT (as alternative imaging study to the standard contrast-enhanced CT)

Bone marrow biopsy and aspirate

Required in cutaneous lymphomas with intermediate to aggressive clinical behavior as categorized in the WHO-EORTC classification

Should be considered in cutaneous lymphomas with indolent clinical behavior, but not required unless indicated by other staging assessments

Additional studies as indicated clinically

*Lymph nodes that are >1.0 cm in short axis and/or have significantly increased PET activity should be sampled for tissue examination (an excisional biopsy is preferable whenever possible)

ANNEXURE IV

Patient Information sheet

Study Title:

Clinicopathological features of cutaneous lymphomas and true prevalence of mycosis fungoides by using T-cell receptor gene rearrangement.

Purpose of research:

We are going to study the signs and symptoms of cutaneous lymphoma (a cancer that affects the skin), the pathology which includes the type of cells in the affected skin with special methods which will be helpful in making a decision regarding treatment and the way in which the disease will affect you.It is difficult to differentiate early stages of mycosis fungoides from common benign dermatoses (non-harmful) by only the signs, symptoms and its pathology. So we are also doing a new test called TCR gene rearrangementin those who have patches which will help us to differentiate mycosis fungoides from non-harmful benign skin diseases. Thus we will be able to make a definite diagnosis and start early treatment.

Expected duration of the Subject's participation:

You will be examined by the doctor only once in the study period.

Description of the procedures:

The doctor will do detailed clinical examination and note down the data in a special proforma. Skin biopsy will be done from the skin lesion for diagnostic purpose which is a part of routine management. Other blood tests and imaging studies will be done as per standard protocol to see if the disease has spread to other extracutaneous sites. TCR gene rearrangement will be done in patients with early stage of mycosis fungoides which will be done from the earlier biopsied tissue or a fresh biopsy tissue.

Risks or discomforts to the Subject:

Skin biopsy will be done for the TCR gene rearrangement. Possible complications include

- Pain
- Bleeding at the biopsy site
- There is also a slight possibility of infection or fainting.
- Post procedure scar

We are not doing any additional studies except TCR gene rearrangement study apart from the standard protocol

Benefits to the Subject:

We are going to study the signs and symptoms of the disease, the pathology and also the type of cells in the affected skin with special techniques which will be helpful in making a decision regarding treatment and the way in which the disease affects you. At present histopathology and study of the type of cell in the skin is used to diagnose all cases of lymphoma. In this study we are going to an additional genetic testing on the skin biopsies of those patients with patches which will help us to differentiate from other skin conditions that can be mistaken for mycosis fungoides.

Benefits to others:

Overall data about cutaneous lymphomas are sparse in India. This study will provide data on prevalence of various types of lymphomas in India and their clinicopathological and immunophenotypical correlating features. By this study we may know the usefulness of TCR gene rearrangement study in distinction of early stage mycosis fungoides from other benign dermatoses.

Confidentiality:

Patient's identity will not be revealed in any information or released to third parties or published. We will keep your records confidential unless we are required by law to share any information.

Participation:

Your participation in the study is entirely voluntary and the patient is free to withdraw at any time, without giving any reason. Refusal to participate will not involve any penalty or loss of benefits to which the subject is otherwise entitled.

Contact person:

Dr. Venkatraman.M,
Post Graduate resident,
Department of Dermatology,
Christian Medical College and Hospital,
Vellore.
Mobile- 9894176756
Office-04162283527

ANNEXURE V

தகவல் படிவம்

ஆராய்ச்சியின் தலைப்பு:

தோல் புற்றுநோயின் (லிம்.்.போமா) நோய் கூற்றியலை நுண்ணாய்வின் முலம் ஆராய்ந்து அறிந்து கொள்ளுதல் மற்றும் மைகோசிஸ் பங்.்.கோயிடஸ் நோயின் திட்டு நிலையை டி.சி.ஆர் (TCR) மரபணு சோதனை மூலம் அறிந்து கொள்ளுதல்.

ஆராய்ச்சியின் நோக்கம் :

ஓர் உயர்நிலை மருத்துவமனையின் தோல் மருத்துவரிடம் வரும் தோல் புற்றுநோயின் தன்மைகள் மற்றும் அதன் கூற்றியியலை அறிந்துக் கொள்ளும் பொருட்டு இவ்வாராய்ச்சி நடத்தப்படுகின்றது. மைகோசிஸ் பங்.்.கோயிடஸ் என்னும் தோல் புற்று நோயின் ஆரம்ப நிலையை தோல் கூற்றியியலின் மூலம் மட்டும் அறிந்து கொள்வது மருத்துவ ரீதியில் மிகவும் கடினமானதாகும். ஆகவே நாங்கள் பாதிக்கப்பட்ட தோல் திசுவிலிருந்து டி.சி.ஆர் (TCR) மரபணு சோதனையின் மூலம் நோயின் ஆரம்ப நிலையை உறுதி செய்வோம். இச்சோதனையை செய்வதன் மூலம் நோயை முன்னரே கண்டறிந்து சிகிச்சையளிக்க முடியும். இம்மரபணு சோதனைக்கான செலவு சற்று அதிகம் என்றாலும் அதனால் ஏற்படும் நன்மைகள் மிக அதிகம்.

நோயாளி பங்கேற்கும் காலம்:

ஆராய்ச்சிக்காலத்தில் பரிசோதிக்கும் மருத்துவரால் நீங்கள் ஒருமுறை மட்டுமே பரிசோதிக்கப்படுவீர்கள். இவ்வாராய்ச்சிக்காக நீங்கள் மறுமுறை அழைக்கப்படமாட்டீர்கள்.

செய்முறை விளக்கம்:

உங்களை பரிசோதிக்கும் மருத்துவர் உங்களின் நோய் தொடர்பான தகவல்களை அதற்கு உரித்தான படிவத்தில் நிரப்பிக் கொள்வார். நுண்ணாய்விற்காக தோல் திசு எடுக்கப்படும். இரத்த சோதனைகளும், நுண்கதிர் சோதனைகளும் தேவைப்பட்டால் செய்யப்படும். இவை அனைத்துமே நோயின் தன்மைகேற்ப செய்யப்படும். இவ்வாராய்ச்சிக்காக எந்தவொரு கூடுதல் சோதனைகளும் செய்யப்படமாட்டாது.

ANNEXURE VI

सूचना पत्र

स्टडी शीर्षक : चर्म लिंफोमा में क्लिनिकल व पैथोलोजिकल विशेषताएँ व माइक्रोबीयल कंगोडड्स चैंप चरण में टी सेल पुर्नव्यवस्था का अध्ययन

1. इस अनुसंधान का लक्ष्य है :-

हम आपसे तृतीयक देखभाल केंद्र में रोग चर्म लिंफोमा की क्लिनिकल विशेषताओं, पेथोलीजीकल व इम्मोनो फिनोटीपीकल संबंध का अध्ययन कर रहे हैं जो कि बीमारी के प्रबंधन व अपेक्षित परिणाम में उपयोगी होगा। इस बीमारी की प्रारंभिक अवस्था को रवायती पेथोलोजिकल व इम्युनोपेथोलोजिकल अध्ययन द्वारा अन्य चर्म रोगों से अंतर करना मुश्किल है इसलिए टी सेल पुर्नव्यवस्था की सहायता ली जाएगी। हालांकि ये परीक्षण महंगा है किंतु इस बीमारी की प्रारंभिक अवस्था के इलाज में यह उपयोगी है एवं इससे मरीज की जीवन प्रत्याशा में सुधार होगा।

2. स्टडी का समय -

इसमें रवायती एक बार डॉक्टर द्वारा आपका परीक्षण किया जाएगा।

3. प्रक्रिया का विवरण -

डॉक्टर आपकी विस्तृत क्लिनिकल जाँच करेगा व इसको एक विशेष प्रोफोर्मा में नोट करेगा। चर्म रोग की जगह से एक बायोप्सी की जाएगी जो कि नियमित जाँच प्रक्रिया का हिस्सा है।

रक्त परीक्षण व इमेजिंग भी विशेष प्रोटोकोल के तहत की जाएगी अगर रोग त्वचा के अलावा किसी और जगह फैल गया है।

ही सेल्ल जीन पुर्नव्यवस्था का अध्ययन पुरानी बायोप्सी से न कि एक और नयी बायोप्सी से किया जाएगा।

4. मरीज के विए जोखिम:

चर्म कैंसर की जाएगी जिसके कारण मरीज को ये कष्ट हो सकते हैं —

(I) दर्द

(II) बायोप्सी के जगह पर रक्तस्राव

(III) संक्रमण । चक्कर आना

(IV) प्रक्रिया के बाद निशान

5. मरीज को लाभ

ही सेल्ल पुर्नव्यवस्था का अध्ययन मारकोसीस जेनोडर्मा व चर्म लिंफोमा को अन्य चर्म रोगों से अलग करता है। जिससे मरीज के जीवन प्रत्याशा में भी सुधार होगा।

6. दूसरे को लाभ:

ही सेल्ल पुर्नव्यवस्था से मारकोसीस जेनोडर्मा की प्रारंभिक अवस्था में ही पता चल जाएगा जिससे रोग के निदान में सुधार होगा।

7. पहचान:

मरीज की पहचान गुप्त रखी जाएगी।

8. शामिल होना:

स्वेच्छा से भाग लेना मरीज की इच्छा पर है। किसी भी समय वह बिना किसी कारण के स्वेच्छा से हट सकता है।

9. सम्पर्क सूत्र: डॉ वेंकट रमन, चर्म रोग विभाग, सिद्धिविनायक मेडिकल कॉलेज, बैंगलोर

ANNEXURE VII

Informed Consent form to participate in a clinical trial

Study Title: Study of clinicopathological features of cutaneous lymphomas and t-cell receptor gamma gene rearrangement in patch stage mycosis fungoides

Study Number:

Subject's Initials: _____ Subject's Name: _____
Date of Birth / Age: _____

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression of the Subject/Legally Acceptable Representative:
Signatory's Name: _____ Date:

Signature of the Investigator: _____ Date: ____/____/_____
Study Investigator's Name: _____

Signature of the Witness: _____Date: ____/____/_____
Name of the Witness: _____

ANNEXURE VIII

ஒப்புதல் படிவம்

மருத்துவ ஆராய்ச்சியில் பங்கேற்பதற்கான ஒப்புதல் படிவம்

ஆராய்ச்சியின் தலைப்பு : தோல் புற்றுநோயின் (லிம்போமா) நோய்கூற்றிலை நுண்ணாய்வின் மூலம் ஆராய்ந்து அறிந்துக்கொள்ளுதல் மற்றும் மைகோசிஸ் ஃபங்கோயிடஸ் நோயின் திட்டு நிலையை டிசிஆர் மரபணு சோதனை மூலம் கண்டறிதல்.

ஆராய்ச்சி எண். :

பங்கேற்பவரின் பெயர் :

பிறந்த தேதி / வயது :

(i) நான்..... தேதியில் மேற்கண்ட தகவல் படிவத்திலுள்ள அனைத்து தகவல்களையும் நன்கு படித்து அறிந்துகொண்டேன். இவ்வாராய்ச்சியின் நோக்கத்தையும் இதில் பங்கெடுத்துக்கொள்வதன் மூலம் ஏற்படும் நன்மைகளையும் அறிந்துகொண்டேன்.

(ii) இவ்வாராய்ச்சியல் பங்கு கொள்வது என் விருப்பம் சார்ந்தது என்பதனையும். இவ்வாராய்ச்சியில் இருந்து எப்போது வேண்டுமானாலும் எக்காரணமும் இன்றி விலகிக்கொள்ளலாம் என்பதனையும் அறிந்து கொண்டேன். என்னுடைய விலகல் என் மருத்துவ சிகிச்சைக்கான எந்தவொரு உரிமையையும் பாதிக்காது என்பதையும் அறிவேன்.

(iii) இவ்வாராய்ச்சி சம்பந்தமான பொறுப்பில் உள்ளவர்கள். சட்டப்பூர்வமான குழுவை சார்ந்தவர்கள் மற்றும் ஒழுங்குமுறை குழுவை சார்ந்தவர்கள் மருத்துவ பதிவேடுகளை என்னுடைய அனுமதியின்றி கையாளலாம் என்பதற்கு எனது சம்மதத்தை தெரிவித்துக்கொள்கிறேன். இதில் எனக்கு எந்த மறுப்பும் இல்லை.

(iv) இவ்வாராய்ச்சியின் தகவல்கள் மற்றும் முடிவுகள் அறிவியல் சம்பந்தமாக பயன்படுத்தபடுவதில் எனக்கு எந்த மறுப்பும் இல்லை.

(v) நான் இவ்வாராய்ச்சியில் பங்குகொள்ள முழுமனதுடன் சம்மதிக்கிறேன்.

நோயாளி / சட்டபூர்வ அனுமதி அளிக்கப்பட்டவரின் கையொப்பம் / கைரேகை

நோயாளியின் பெயர் :.....தேதி :.....

பரிசோதிப்பவரின் பெயர் :.....

சாட்சியாளரின் கையொப்பம் / பெயர் : .....

தேதி : .....

ANNEXURE IX

क्लिनिकल स्टडी में भाग लेने हेतु अनुमति पत्र

स्टडी शीर्षक : चर्म लिंफोमा में क्लिनिकल व पेथोलोजिकल विशेषताएँ व पैंच चरण मारकोसौस फंगोइड्स में टी सेल जीन पुर्नव्यवस्था का अध्ययन

स्टडी नम्बर -

मरीज का नाम -

जन्मतिथि / उम्र -

1. मैंने उपरोक्त स्टडी के सूचना पत्र को पढ़ व समझ लिया है व मुझे इससे सम्बन्धित प्रश्न पूछने का मौका दिया गया।

2. मैं जानता / जानती हूं कि स्टडी में भाग लेना मेरी इच्छा पर है तथा किसी भी समय मैं इसमें हट सकता / सकती हूं बिना किसी कारण के व बिना मेरी चिकित्सा व कानूनी अधिकार प्रभावित हुए।

3. मैं जानता हूं कि क्लिनिकल स्टडी के स्पोन्सर व उससे सम्बन्धित लोग व इथिक्स कमेटी, रेगुलेटरी अथोरिटी को मेरा स्वास्थ्य रिकॉर्ड इस स्टडी व अनुसंधान में शामिल करने के लिए मेरी अनुमति की जरूरत नहीं है। मैं जानता हूं कि मेरी पहचान गुप्त रखी जाएगी।

4. मैं इस स्टडी के परिणाम व किसी अन्य तथ्य के वैज्ञानिक अध्ययन के लिए सहमत हूं।

5. मैं उपरोक्त स्टडी में भाग लेने के लिए सहमत हूं।

हस्ताक्षर (अंगूठे का निशान)

मरीज / मरीज के कानूनी संरक्षक

दिनांक

हस्ताक्षर कर्ता का नाम

जाँच कर्ता के हस्ताक्षर

दिनांक

जाँच कर्ता का नाम

गवाह के हस्ताक्षर

दिनांक

गवाह का नाम

ANNEXURE X

X(A).TECHNIQUE OF MANUAL IMMUNOHISTOCHEMISTRY BY ENVISION/2 STEP POLYMER METHOD.

1. Approximately three microns thick sections were cut and floated on pol y-lysine coated slides and incubated 37 degrees centigrade overnight. An appropriate control was used for the antibody tested.
2. Sections were dewaxed in xylene for 15 minutes and brought to water.
3. Antigen retrieval was done by heat induced pressure cooking

a. Slides depending on the IHC antibody used were placed in the containercontaining either citrate buffer at pH 6 or EDTA buffer at pH 9 and placed in the cooker with lid closed for 30seconds at a temperature of 120 degrees centigrade and a pressure of 15 psi

b. After boiling, the cooker was cooled by quenching in a sink of cold running tap water.

4. The slides were removed from the cooker, quickly washed in running tap water and transferred to TRIS buffered saline {TBS} at pH 7.6
5. 0.3% Hydrogen peroxide (H2O2) was added to the slide and incubated at room temperature for 10 min to block endogenous peroxidase.
6. The slides were washed in TBS at pH 7.6
7. The primary antibody was applied and incubated at room temperature for 30 minutes.
8. The slides were washed in TBS at pH 7.6
9. Envision (commercially available secondary antibody + enzyme) was applied and incubated at room temperature for 30 minutes.
10. The slides were washed in TBS at pH 7.6
11. Chromogen (Diaminobenzidine) along with substrate (hydrogen peroxide) was applied.
12. Slides were washed in running tap water.
13. The slides were counterstained with Harris haematoxylin for 10 seconds and then dehydrated, cleared and mounted.

ANNEXURE X (B)

PREPARATION OF REAGENTS USED FOR IMMUNOHISTOCHEMISTRY

1. TBS {TRIS from Aldrich}. The pH is checked and adjusted to 7.6

Nacl	80g
TRIS	6.05g
1N HCl	40ml
Distilled H2O	10 litres

2. Hydrogen peroxide – H_2O_2 is available commercially as 30% solution fromQualigens{SQ}. A 0.3% solution is prepared by adding 1 ml of H2O2 to 9 ml of distilled water.

3. DAB solution – Commercially available 5 ml solution from DAKO along with 250 ml of substrate buffer. Working solution is prepared by mixing DAB solution and substrate buffer in a ratio of1:50.

ANNEXURE XI

MULTIPLEX POLYMERASE CHAIN REACTION ASSAY FOR T-CELL RECEPTOR GAMMA CHAIN GENE REARRANGEMENT

SUMMARY

This test is indicated for the accurate differentiation of malignant vs benign lymphoproliferative disorders and for establishing T cell lineage. Clonal proliferations of T lymphocytes can be detected by the identification of specific DNA rearrangements in the T-cell gamma chain antigen receptor gene. The majority of lymphocytic leukemias and non-Hodgkin's lymphomas arise from a clonal proliferation of a single lymphoid cell that has become aberrant. T cells normally differentiate from stem (precursor) cells in a highly specific and sequential manner. The malignant transformation may take place at any stage in the maturation process and when it occurs, the resulting malignant clone bears the characteristics of the originally transformed cell and is called monoclonal. Some of the early events in the maturation process of lymphoid cells involve specific nucleic acid rearrangements within the gamma chain antigen receptor gene in T-cells.

T-cell gene rearrangement testing can be used to determine the presence of a monoclonal T cell population in a submitted sample, indicative of a neoplastic lymphoproliferative disorder. Southern blot testing using genomic probes to the beta chain antigen receptor gene has been the method of choice for establishing the monoclonal nature of a T lymphoid process. This test, however accurate, requires fresh frozen tissue and at least 20 ug of high molecular weight DNA to perform accurately. In cases where there is not a sufficient amount of tissue (or the tissue has been poorly preserved), the Polymerase Chain Reaction (PCR) can be utilized to detect the clonal population. PCR also does not required fresh or frozen tissue and can be used on fixed and paraffin embedded material.

Polymerase Chain Reaction (PCR) refers to in-vitro enzymatic amplification of a defined DNA sequence by repeated rounds of heat denaturation, specific DNA primer annealing, and DNA polymerase-mediated primer extension. The use of a thermostable DNA polymerase found in the bacteria Thermusaquaticus allows for repeated rounds of amplification without further addition of polymerase enzyme at each cycle. Each cycle causes a doubling of the specific sequence circumscribed by the primers so that production of specific DNA product ideally approaches 2^n where n = the number of amplification cycles**.**

A. PRINCIPLE

To detect T cell gene rearrangements using PCR, a multiple pairs of primers are constructed that target multiple exons with in the variable (V) region and the joining (J) of the T cell gamma chain antigen receptor gene. In the germline configuration found

in somatic cells, these areas are widely separated (by more than 70KB) making amplification across the area impossible. The sequence alteration brought about by gene rearrangement brings these regions into close proximity, making the area of amplifiable length. Each T cell has a single productive V-J rearrangement that is unique in both length and sequence. The template-free incorporation of nucleotides (N-regions) between the spliced V and J gene segments also adds uniqueness to the PCR product. Therefore, when this region is amplified using DNA primers that flank this region, a clonal population of cells yields one or two prominent amplified products (amplicons) within the expected size range. Two products are produced in cases when the initial rearrangement was non-productive and was followed by rearrangement of the other homologous chromosome. In contrast, DNA from a normal or polyclonal population produces a bell-shaped curve (or Gaussian distribution) of amplicon products that reflects the heterogeneous population of V-J region rearrangements.

Since the antigen receptor genes are highly polymorphic, it is difficult to employ a single set of DNA primer sequences to target all of the conserved flanking regions around the V-J rearrangement. N-region diversity and somatic mutation further scramble the DNA sequences in these regions. Therefore multiplex master mixes, which target several regions (Vgamma 1-8,9 + J1/2 consensus for T1 and Alternate V gamma + J1/2 for T2), are required to identify the majority of clonal rearrangements. As indicated, clonal rearrangements are identified as prominent single-sized products within the smear of different sized amplicon products that form a Gaussian distribution around a statistically favored, average-sized rearrangement. As expected, primers that amplify from the different gamma chain regions produce correspondingly different size ranges of products.

A powerful detection method for the amplicons produced in the multiplex reaction involves capillary electrophoresis and differential fluorescence detection of the amplified products. The primers used in the master mixes are labeled with different fluorescent dyes, each corresponding to a different target region (V-J combinations). This detection system results in unsurpassed sensitivity, single base resolution, differential product detection and relative quantitation. Further, differential detection allows accurate, reproducible and objective interpretation of primer-specific products and automatic archiving of data. The limit of detection of this assay has been determined to be approximately 1 clonal cell in 100 normal cell. The inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1-2 base pairs. This reproducibility and sensitivity allows monitoring and tracking of individual tumors. The automatic archiving of specimen data allows the flexibility to compare data collected at different times.

B. SPECIMEN REQUIREMENTS

1. TYPE

Analysis can be performed on a variety of tissues which include whole blood (1 ml), bone marrow aspirate 0.5 ml) or biopsy (approx. 1 cm), solid tissue (0.1 x 0.1 x 0.1 cm) or aspirated cells ($1x10^6$ minimum), specimens embedded in paraffin (see below). Samples for T Cell PCR are forwarded from samples that did not yield conclusive results from flow cytometry evaluation (body fluids or tissue) or directly from external clients. Samples may be forwarded to the laboratory in the fresh state, frozen, or as paraffin embedded sections.

2. HANDLING AND STORAGE CONDITIONS

All specimens should be handled according to Standard Precautions guidelines established by the University (see section on Blood borne Pathogens) Policy & Procedure Manual).

a) Whole blood and marrow aspirates should be collected in EDTA or ACD and refrigerated, but NOT FROZEN. Samples collected in Heparin are not suitable for PCR analysis.
b) Bone marrow biopsies should be placed in a container without media and frozen.
c) Solid tissues should be trimmed free of fat, connective tissue, and excess normal tissue and FROZEN at -30^{o}C or colder.
d) Skin biopsies for the diagnosis of CUTANEOUS LYMPHOMAS requires a minimum of 1 punch biopsy (4 mm) of the area involved. This should be placed in a container without media and frozen.
e) Cells in suspension should be centrifuged, the supernatant decanted, and frozen as a pellet.
f) Specimens fixed in 95% ethanol may be submitted.
g) Samples embedded in OTC medium may be submitted.
f) For specimens embedded in paraffin, sections should be cut and placed dry in a 1.5 ml PCR screw cap micro centrifuge tube. (see General Policies & Procedure Man., Specimen Handling Protocol Sec. A., Clonality Testing by PCR Specimen Handling # 3-4). Paraffin embedded tissue is stored at room temperature until DNA is extracted. Once DNA is extracted, it can be stored at -20C. All specimens should be cut using PCR cross-contamination precautions. These include using a new knife for each different block being cut and cleaning all contact surfaces with xylene to remove any possible contamination from other blocks.

a) If the tissue is 0.5 x 0.5 cm or larger, (five) 5 x 10u sections should be cut and placed dry in a 1.5 ml PCR screw cap micro centrifuge tube.
b) If the tissue is smaller than 0.5 x 0.5 cm, (ten) 10 x 10u sections should be cut and placed dry in a 1.5 ml PCR screw cap micro centrifuge tube.
c) For skin biopsies (3 or 4mm punch bx), (twenty) 10 x 20u sections should be cut and placed dry in a 1.5 ml PCR screw cap micro centrifuge tube.
d) Specimens fixed in B5 fixative are not suitable for PCR analysis.

3. REJECTION CRITERIA

Samples not appropriately labeled with patient name will be rejected and returned to the requestor at the discretion of the Lab Director. Due to the delicate and valuable nature of many of the samples, efforts will be made to verify identity and integrity**.**

C. EQUIPMENT / REAGENTS / MATERIALS

EQUIPMENT

Dubnoff Shaking Water Bath
Fisher Micro centrifuge Model 59A
Gilson Pipetman pipettes (P10, P20, P200, P1000)
Aerosol barrier pipet tips for the Pipetman pipettes
Perkin Elmer GeneAmp 9600 PCR thermal cycler or ABI GeneAmp PCR System 9700.
ABI 3100-Avant Genetic Analyzer and related consumables.
ABI 3100-Avant Capillary Array (36 cm)
National Appliance Vacuum Oven
Screw-cap, 1.5 ml micro centrifuge tubes w/ cap inserts
500 ulmicrocentrifuge tubes-assorted colors
MicroAmp tubes with attached caps or strips (8) and related cap strips (ABI or compatible)
Lab-Line Multi-Blok heating block

MATERIALS AND STORAGE REQUIREMENTS

Reagents-General Considerations

The lot number of all critical reagents used in these procedures (as stated in the Validation of Reagents, General Policies & Procedures Manual) should be noted in the Molecular Biology Reagent log book when they are put in use. All reagents should be dated when they are received, opened or placed in use, and expiration date noted (if applicable). All laboratory chemicals (salts, solvents, acids, bases, etc.) in original manufacturer's containers have no applicable expiration date unless otherwise noted on the container by the manufacturer. All reagents must be stored under the specifications given by the manufacturer or as otherwise noted below. All laboratory-prepared reagents must be appropriately labeled with name, concentration if applicable, date prepared, storage conditions, expiration date, and tech initials. No reagent is used past its noted expiration date.

XYLENE

Xylenes (for de-paraffination of sections). (Mallinckrodt Chemical Co.) Store at room temperature. Do not use near heat or open flame. No expiration date.

ETHANOL

100% Absolute ethyl alcohol, 200 proof (Florida Distillers Corp.) Store at room temperature. Do not use near heat or open flame. No expiration date.

QIAGEN QIAamp DNA MINI KIT

QIAGEN Catalogue #51304.Store at room temperature until opening. Good until manufacturer's expiration date if properly stored. The kit contains the following components that have their own storage conditions after opening.

Qiagen Buffer ATL

For tissue lysis.Store at room temperature. Mix thoroughly by shaking before use. Good until manufacturer's expiration date if properly stored.

Buffer AL

Mix thoroughly by shaking before use. Stable for 1 year at room temperature.

Buffer AW1

Add 25 ml of absolute ethanol to buffer AW1 concentrate before use. Final volume for is 44ml.

Store closed at room temperature. Stable for 1 year after reconstitution if appropriately stored.

Buffer AW2

Add 30 ml of absolute ethanol to buffer AW2 concentrate before use. Final volume for is 43ml.

Store closed at at room temperature. Stable for 1 year after reconstitution if appropriately stored.

QIAamp Spin Columns

Store at room temperature in sealed plastic bag. Do not mix lot numbers of columns. Good until manufacture's expiration date if appropriately stored.

Qiagen Buffer EL

Purchased separately for lysis of red blood cells.Store at room temperature until opening. Good until manufacturer's expiration date if properly stored.

RED CELL LYSIS BUFFER

10mM Tris (pH 7.5), 5mM MgCl2, 0.32 M Sucrose, 1% Triton X-100. Add 109.5g Sucrose to 500 ml dH2O and stir until dissolved. Add 1.21g Tris base and either 1.02g of $MgCl_2$ crystals or 5ml of 1M $MgCl_2$ solution (Sigma Chem. Co.). Adjust pH to 7.5 with HCl, add 10 ml Triton X-100, and QS to 1 liter. Store at 4^0C. Good for 18 months if properly stored.

PROTEINASE K

Proteinase K MBG (Promega Scientific) stored at -20^{0}C. Working solution is 50mg/ml in distilled H_2O. Aliquot and store at -20^{o}C. Good for 6 months if appropriately stored.

AMPLITAQ GOLD DNA POLYMERASE

Recombinant, thermostable, heat activated 94kDa DNA polymerase derived from Thermusaquaticus DNA polymerase gene and inserted into E. coli (Perkin Elmer). 5U/ul, 250U/vial. Store frozen at -20^{0}C in a constant temperature freezer. Good until manufacturer's control date on tube if properly stored.

T CELL RECEPTOR GAMMA GENE REARRANGEMENT ASSAY KIT

Invivoscribe Technologies, San Diego, CA.Catalogue # 1-207-0011.Contains fluorescent-labeled PCR reagents for identification of clonal T cell popluations. Store frozen at -20^{0}C in a constant temperature freezer. Good until manufacturer's control date on individual reagent tubes if properly stored (Invivoscribe Technologies, San Diego, CA). The kit contains the following components that after opening must be stored in the manufacturer's recommended storage conditions. Only the following components are used in the Multiplex T Cell Receptor Gamma Gene Rearrangement PCR assay:

T Cell Receptor Gamma Master Mix 1

Catalogue # 2-207-0011. Contains multiple primer sequences that target the Vgamma 1-8,9 + J1/2 consensus region of the T gamma chain locus. All other required reactants to ensure robust amplification of the target region (i.e. PCR buffer, salts and dNTP bases), excluding the Amplitaq DNA polymerase, are included in the master mix. For undiluted use only. Store frozen at -20^{0}C in a constant temperature freezer. Good until manufacturer's control date on tube if properly stored.

T Cell Receptor Gamma Master Mix 2

Catalogue # 2-207-0021.Contains multiple primer sequences that target alternate V gamma + J1/2 regions of the T gamma chain locus. All other required reactants to ensure robust amplification of the target region (i.e. PCR buffer, salts and dNTP bases), excluding the Amplitaq DNA polymerase, are included in the master mix. For undiluted use only. Store frozen at -20^{0}C in a constant temperature freezer. Good until manufacturer's control date on tube if properly stored.

Specimen Control Size Ladder

Catalogue # 2-096-0021.Contains primers that target multiple human housekeeping genes and generates a series of amplicons of 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input sample DNA is adequate to yield a valid result.For undiluted use only. Store frozen at -20^{0}C in a constant temperature freezer. Good until manufacturer's control date on tube if properly stored.

Clonal T Cell DNA (JURKAT)

Catalogue # 4-200-0060. 100ul of Positive control Human DNA that contain detectable clonal rearrangements in the T gamma chain gene region described at a concentration of 200ug/ml. For undiluted use only. Store frozen at -20^{0}C in a constant temperature freezer. Good until manufacturer's control date on tube if properly stored.

Human Polyclonal Genomic DNA
Catalogue # 4-101-0010. 100ul of Negative control Human DNA that does not contain detectable clonal rearrangements in the specific T gamma antigen receptor gene regions described at a concentration of 200ug/ml. For undiluted use only. Store frozen at -20^{0}C in a constant temperature freezer. Good until manufacturer's control date on tube if properly stored.

HI-DI ^{TM} FORMAMIDE

Catalogue # 4311320.De-ionized formamide. Store frozen at -20^{0}C in a constant temperature freezer prior to opening. After opening, pipet 1 ml aliquots and store in screw-cap microcentrifuge tubes. Store frozen at -20^{0}C in a constant temperature freezer until use. Good until manufacturer's control date on container if properly stored. Applied Biosystems, Foster City, CA.

GENETIC ANALYZER BUFFER (10X) WITH EDTA

Catalogue # 402824. Proprietary buffer for use with ABI 3100-Avant instrument for capillary electrophoresis detection of fluorescent labeled DNA sequences. Store at 2-8^{0}C. Good until manufacturer's control date on container if properly stored. Applied Biosystems, Foster City, CA.

GENESCAN 400HD ^{TM} (ROX) SIZE STANDARD

Catalogue # 402985. Labeled size standards for use with ABI 3100-Avant instrument for capillary electrophoresis detection of fluorescent labeled DNA sequences. Store at 2-8^{0}C. Alternatively, may be dispensed (40ul) into 1 ml aliquots of HI-Di Formamide reagent and stored frozen at -20^{0}C until use. Good until manufacturer's control date on container if properly stored. Applied Biosystems, Foster City, CA.

3100 POP-4^{TM} PERFORMANCE OPTIMIZED POLYMER

Catalogue # 4316355. Gel polymer optimized for use in the 3100-Avant Genetic Analyzer for capillary electrophoresis detection of fluorescent labeled DNA sequences. Store at 2-8^{0}C. Good until manufacturer's control date on container if properly stored. Applied Biosystems, Foster City, CA.

D. QUALITY CONTROL

1. CONTROLS FOR REAGENTS

The lot number of all critical reagents used in these procedures (as stated in the Validation of Reagents, General Policies & Procedures Manual) should be noted in the Molecular Biology Reagent logbook when they are put in use. All new critical reagent lots (as stated in the Validation of Reagents, General Policies & Procedures Manual)

are tested against the lot currently in use to assess functionality prior to use on clinical samples.

2. CONTROL OF AMPLIFIABLE DNA (SAMPLE): SPECIMEN CONTROL SIZE LADDER

All samples are analyzed using the Specimen Control Size Ladder amplification master mix (Size Ladder) to determine the presence of 'amplifiable DNA'. The Size ladder amplification reaction will yield amplicons of 89bp, 96bp, 200bp, 300bp, 400bp, and 600bp with human DNA specimens. For a patient result to be considered valid, the sample DNA must show peaks in the size ladder control larger than the upper size limit of the valid size range result for the particular master mix being evaluated.

3. CONTROLS FOR T CELL CLONALITY

Each run of multiplex T cell PCR must include a No Template Control (NTC), a Negative Control (Human Polyclonal Genomic DNA) and a Positive Control (Clonal T Cell (JRUKAT) Positive Control DNA). To consider the run valid, the No Template Control (NTC) must show no major peaks, in the valid size range for each of the master mixes used (Tubes 1 and 2). To consider the run valid, the Negative Control must show a Gaussian distribution of peaks, and no specific monoclonal peaks, in the valid size range for each of the master mixes used (Tubes 1 and 2). To consider the run valid, the positive control must show a specific peak or peaks of the correct size in each specific master mix tested.

4. CONTROL FOR ACCURACY AND PRECISION

Each run shall include the Clonal T Cell (JURKAT) Positive Control DNA in the run. This control is evaluated by the Lab Director prior to evaluating patient results. The result for the positive control must fall in the expected control range (expected size in bp +/- 5bp) to be considered valid. These results will be plotted and evaluated monthly for any size variation trend. Each run shall include a negative control (Human Polyclonal Genomic DNA). This control is evaluated by the Lab Director prior to evaluating patient results.

E. PROCEDURE-STEPWISE

DNA SAMPLE PREPARATION (Qiagen kit procedure for blood and body fluids)

1. Remove all blood and body fluid samples for analysis from the refrigerator and allow to come to room temperature. Log them into the DMPL specimen log book giving each sample a unique, consecutive identifying number. This number will be used to identify the sample throughout the rest of the procedure. Centrifuge the samples at 3000 RPM for 10 minutes.

2. Prepare a water bath at 56^0 C.

3. Prepare two 1.5 ml screw-cap microcentrifuge tubes per sample. Label each tube with the unique sample identifying number (i.e DMPL#1000 PCR). Prepare one spin column and three 2 ml collection tubes per sample. Label just the spin column with the DMPL unique identifying number. For each sample prepare final spin collection tube by taking a 1.5 ml microcentrifuge tube and cutting off the cap end. Do not label any of the collection tubes.

4. Remove sample tubes from centrifuge. For each blood specimen, open the tube, pipet out the plasma , and discard. Carefully remove the buffy coat cells and place into appropriately labeled 1.5 ml screw-cap microcentrifuge tubes. Add at least an equal volume of Buffer EL. Invert the tube several times until all red cells are lysed (sample becomes translucent).

5. For body fluid samples, pipet excess supernatant leaving approximately 200 ul residual supernatant in tube. Proceed to step #7.

6. Centrifuge microcentrifuge tubes for 30 seconds at full speed. Decant the supernatant carefully. Add sufficient saline to bring the volume left in the sample tube to 200ul. Vortex the tube containing the remaining cells and saline, making sure to break up any pellet, prior to continuing with the next step.

7. Perform the following steps to each sample <u>individually</u>. Add 25ul Proteinase K solution to the sample tube and mix well with sample. Add 200 ul of Buffer AL to the sample and <u>immediately</u> mix thoroughly by pulse vortexing for at least 15 seconds to break up any pellet.

8. Incubate tubes at 56^0 C for 10 minutes. Remove from water bath and briefly centrifuge to bring all liquid to the bottom.

9. Add 200 ul of absolute ethanol to the sample and mix thoroughly by vortexing. It is normal for a fibrous, white precipitate to form inside the sample mixture. Briefly centrifuge to bring all liquid to the bottom.

10. Place the appropriately labeled QIAamp spin column in a 2 ml collection tube. Carefully transfer the entire contents of the sample tube to the spin column including any fibrous precipitate that may have formed with the EtOH addition. Close the cap on the column. When all the samples have been processed, place the tubes in the microcentrifuge and centrifuge at full speed for 2 minutes until all mixtures have passed through the columns.

11. Place each QIAamp spin column in a clean 2 ml collection tube and discard the tube containing the filtrate. Carefully open each QIAamp spin column and add 500 ul of Buffer AW1. Close the cap on the column. When all the samples have buffer AW1 and are capped, place the tubes in the microcentrifuge and centrifuge at full speed for 2 minutes until the wash solution has passed through all the columns.

12. Place each QIAamp spin column in a clean 2 ml collection tube and discard the tube containing the filtrate. Carefully open each QIAamp spin column and add 500 ul of Buffer AW2. Close the cap on the column. When all the samples have buffer

AW2 and are capped, place the tubes in the microcentrifuge and centrifuge at full speed for 3 minutes until the wash solution has passed through all the columns.

13. Place the QIAamp spin columns in clean 1.5 ml microcentrifuge tubes originally prepared in step 3 and discard the collection tube containing the filtrate. Add reagent water (50 ul for body fluids, 100 ul for blood) to the QIAamp spin column. Incubate at room temperature for 5 minutes. Place the tubes in the microcentrifuge and centrifuge at full speed for 2 minutes. This step elutes the DNA from the column.

14. Remove the spin column from the 1.5 ml microcentrifuge collection tube and transfer the eluate into the second uniquely labeled 1.5 ml screw capped microcentrifuge tube from step 3. Repeat step #13 and combine eluates.

15. For body fluids that had no visible pellet upon initial centrifugation, the eluate may be concentrated by placing the microcentrifuge tube containing the eluate open in a heated vacuum oven for 5 minutes. Evaporate the eluate to approximately 50 ul remaining in the tube. Proceed with PCR analysis.

DNA SAMPLE PREPARATION (Qiagen tissue method)

1. All samples for PCR are labeled with the DMBL ID #. If sample is fresh frozen tissue, place in PCR screw cap tube and proceed to step #8. If sample is paraffin embedded tissue, add 950 ul of xylene to PCR screw cap tube containing the paraffin embedded tissue. Vortex vigorously to dissolve the paraffin.

2. Centrifuge for 2 minutes at maximum speed in Fisher Microcentrifuge to pellet tissue.

3. Aspirate xylene using micropipette and repeat steps 1 (except using 500 ul of xylene) and 2. Aspirate xylene

4. Add 500ul of absolute ethanol. Vortex vigorously to remove xylene.

5. Centrifuge for 2 minutes to pellet tissue. Decant the ethanol.

6. Repeat steps 4 & 5. Decant the ethanol.

7. Gently dislodge tissue pellet from bottom of tube by inverting or with clean pipet tip press pellet onto side of tube. Dry the tissue pellet in a warm vacuum oven until completely dry (approx. 15 min.).

8. Resuspend the tissue pellet in ATL buffer depending on size of tissue pellet. Add 180 ul ATL buffer for small pellets, 360ul buffer for larger pellets. Add 25 ul of proteinase K per 180 ul ATL buffer and vortex.

9. Screw cap on tightly and incubate with, shaking at, 56^0C in a water bath, vortexing every 15-20 minutes until tissue is completely dissolved. Incubate overnight with

shaking. If necessary, add more Proteinase K. Overnight incubation is absolutely essential for paraffin-embedded tissue specimens.

10. Remove tubes from shaking water bath and centrifuge briefly to remove drops from inside of tube.

11. Add 200 ul of Buffer AL (per 180 ul of buffer ATL added to the sample) and mix by pulse vortexing. Incubate at 70^0C for 10 minute. Briefly centrifuge the tubes to remove drops from inside of tube.

12. Add 200 ul of absolute ethanol to the sample and mix thoroughly by vortexing. It is normal for a fibrous, white precipitate to form inside the sample mixture. Briefly centrifuge to bring all liquid to the bottom.

13. Place the appropriately labeled QIAamp spin column in a 2 ml collection tube. Carefully transfer the entire contents of the sample tube to the spin column including any fibrous precipitate that may have formed with the EtOH addition. Close the cap on the column. When all the samples have been processed, place the tubes in the microcentrifuge and centrifuge at full speed for 2 minutes until all mixtures have passed through the columns.

14. Place each QIAamp spin column in a clean 2 ml collection tube and discard the tube containing the filtrate. Carefully open each QIAamp spin column and add 500 ul of Buffer AW1. Close the cap on the column. When all the samples have buffer AW1 and are capped, place the tubes in the microcentrifuge and centrifuge at full speed for 2 minutes until the wash solution has passed through all the columns.

15. Place each QIAamp spin column in a clean 2 ml collection tube and discard the tube containing the filtrate. Carefully open each QIAamp spin column and add 500 ul of Buffer AW2. Close the cap on the column. When all the samples have buffer AW2 and are capped, place the tubes in the microcentrifuge and centrifuge at full speed for 3 minutes until the wash solution has passed through all the columns.

16. Place the QIAamp spin columns in clean 1.5 ml microcentrifuge tubes originally prepared in step 3 and discard the collection tube containing the filtrate. Add 50 ul reagent water, to the QIAamp spin column. Incubate at room temperature for 5 minutes. Place the tubes in the microcentrifuge and centrifuge at full speed for 2 minutes. This step elutes the DNA from the column.

17. Remove the spin column from the 1.5 ml microcentrifuge collection tube and transfer the eluate into the second uniquely labeled 1.5 ml screw capped microcentrifuge tube from step 3. Repeat step #13 and combine eluates.

18. For tissues that had minimal or no visible pellet upon initial centrifugation, the eluate may be concentrated by placing the microcentrifuge tube containing the eluate open in a heated vacuum oven for 5 minutes. Evaporate the eluate to approximately 50 ul remaining in the tube. Proceed with PCR analysis.

T CELL MULTIPLEX PCR TESTING

1. Remove the appropriate T Cell Gamma Gene Rearrangement Assay kit master mix reagents (tubes T1 and T2) and Size Ladder (SL) master mix reagent and allow to thaw in the PCR reagent preparation area. Remove Kit Negative (Human Polyclonal DNA) and Kit Positive control (Clonal T Cell DNA JURKAT) and allow to thaw in the PCR set up area.

2. Prepare T PCR run log and calculate amount of each master mix required to assay all controls and patient samples. Each run must include a No Template Control (NTC), a Negative Control (KITNEG) and a Positive Control (KITPOS). Each sample or control requires 25 ul of master mix reagent. Each sample or control requires 0.15 ul of Amplitaq Gold DNA polymerase.

3. Prepare working master mixes in the reagent preparation hood by adding Amplitaq Gold enzyme. For each Master mix reaction, add appropriate stock master mix reagent and Amplitaq Gold to labeled working master mix tube and mix well. Spin briefly. Aliquot 25 ul working master mix per PCR tube color-coded for each master mix (T1=Yellow/Brown, T2=Purple, Red= SL) and cap tubes.

4. Add 2.5 ul PCR water to NTC (no template control) control tubes (T1 and T2). Add 2.5 ul Kit positive control to the positive control tubes. Add 2.5 ul Kit Negative (Human Polyclonal DNA) control to the Negative control tubes (T1 and T2). Add 2.5 ul of each patient sample to the appropriate master mix tubes as in the run log T1, T2, and SL). Note, the Size Ladder control reaction is run only on patient samples.

5. Place tubes in the 9600 Thermal cycler or the 9700 Thermal cylcer to amplify. The 9600 is set to run on Method 87. This method consists of a hold file #80, a cycling file #86 and two more hold files #81, and #9. File 80 runs first as follows: 95^0C for 7 min. This file is followed by file 86 which runs as follows: 94^0C for 30 seconds, 55^0C for 30 seconds, 72^0C for 60 seconds for 35 cycles. After the last cycle in file #83, the first hold file # 81 holds samples at 72^0C for 10 minutes and the final hold file #9 holds the samples at 4^0C until the STOP button is pressed. The 9700 Thermal cylcer is set to run the 3100-T-gamma file. It has the same cycling parameters as the Method file #87 in the 9600.

6. When cycling time is over, remove samples from thermal cycler. You can proceed to the next step or store MicroAmp tubes refrigerated until used. Samples can be stored refrigerated for 2 weeks before use in the detection step.

7. Thaw one or more HI-DI Formamide aliquots (1 ml) and add 40 ul of GENESCAN 400HD(ROX) size standard to each if not aliquoted together prior to freezing. Mix thoroughly.

8. Prepare one yellow 500 ulmicrocentrifuge tube for each T1 PCR reaction patient or control sample tube and label them accordingly. Prepare one purple 500 ulmicrocentrifuge tube for each T2 PCR reaction patient or control sample tube and number them accordingly. Add 10ul of HI-DI ROX mixture to each tube. Prepare

one red 500 ulmicrocentrifuge tube for each T PCR patient SL amplification tube and add 10ul of HI-DI/ROX mixture to each tube.

9. Add 1 ul of each amplification reaction product for the patient or control to the color-coded, labeled tube containing the 10ul of Hi-Di/ROX. Add 1 ulof the SL reaction for each patient to the color-coded, labeled tube containing 10ul of HI-DI/ROX mixture.

10. Place tubes in 95^0C heating block for 2 minutes. Remove tubes from the heat block and immediately place in an ice bath for 5 minutes.

11. Prepare a Plate loading record for the 3100-Avant. Place either a 96-well or 384-well plate in the appropriate base and load 10ul of each sample or control as indicated in the Plate Record.

12. Prepare fresh 3100 running buffer by adding 2.5 ml GENETIC ANALYZER BUFFER (10X) reagent and dilute to 25 ml in a graduated cylinder. Mix by inverting. It is now ready for use.

13. The 3100-Avant is a 4 capillary instrument and performs 4 injections at one time, therefore, all 4 wells of the 'quad' injected must be filled. Use water or Hi-Di Formamide to fill any wells of a 'quad' that will not be used in the run.

14. Cover the plate with the appropriate Plate Septa and top with the Plate Retainer. Plate is now ready for loading into 3100-Avant instrument.

15. To load the plate press the 'Tray" button on the front of the 3100-Avant instrument. Do not open the doors until the tray cycles and stops. Open the door and place plate in bay 'B' with the notched end facing the rear of the instrument. The tray is now loaded into the instrument.

16. Remove the Anode Buffer Reservoir jar and remove old running buffer. Rinse with distilled water. Add 10 ml of fresh running buffer and replace gently. Remove Cathode Buffer Reservoir, remove Reservoir Septa and pour out old running buffer. Rinse with distilled water. Add 15 ml of fresh running buffer, replace septa and replace Cathode buffer reservoir in tray assembly. Close 3100-Avant doors. Instrument is now ready to run.

17. Prior to running a plate on the 3100-Avant, a record of samples to be run must be entered into the software. This is called a Plate Record. To create Plate Record in the 3100-Avant, enter Foundation Data collection software and select PLATE MANAGER. Click on NEW and enter plate name-date in the New Plate Dialog Box. Add description if desired. For Application, choose GeneMapper DMP-01, choose appropriate plate type (96 or 384 well), enter Owner and Operator as appropriate and click OK.

18. The PLATE EDITOR screen appears next. Enter specimen ID under the SAMPLE NAME box for the correct WELL # as loaded in the plate loading record. Add comment for sample if desired.

19. Once all samples are entered into the Plate Record, go to SAMPLE TYPE and choose 'Sample' from the drop down menu. Next go to the SIZE STANDARD column, and choose 'GS(400)HD-ROX' as the size standard. Go to the PANEL column and choose 'None'. Go to the ANALYSIS METHOD column and choose 'ServiceFrag'. Go to RESULTS GROUP and choose the appropriate results group (i.e. 'Alex results group'). Go to the INSTRUMENT PROTOCOL column and choose 'IGH4-29-04'. Select all the SAMPLE TYPE, SIZE STANDARD, PANEL, ANALYSIS METHOD, RESULTS GROUP, and INSTRUMENT PROTOCOL columns corresponding to the samples and controls entered in the Plate Record. When so selected, these should appear in blue. Kit the keys 'Control D' to automatically auto-enter the information in all the columns.

20. Click on OK to save the Plate Record. Use the EDIT button to modify the plate record if necessary.

21. Next go to RUN SCHEDULER and double click to open options. Click on 'FIND ALL' and select the correct plate record (it should say 'pending'). Click on the image of the instrument tray bay 'B' (which should be yellow) to link the plate in the bay to the selected plate record. The image of bay 'B' will turn green when the run record is linked. In a few seconds, the RUN button (like a PLAY button on a tape machine) will become active and turn green.

22. Click on the RUN button to start run. The following on-screen message will appear on the computer screen, 'You are about to start processing plates. OK'. Click on OK to initiate run on 3100-Avant.

23. Go to the RUN SCHEDULER and select RUN VIEW. Find the run numbers for the injections in the plate record being processed. Note the run numbers on the Plate Loading Record for future use in retrieving data in GeneMapper.

24. The instrument function and parameters may be monitored by using the INSTRUMENT STATUS screen.

25. Each injection 'quad' takes approximately 35-40 minutes to process. The instrument may be allowed to function during the day or overnight unattended.

DATA REDUCTION USING GENEMAPPER SOFTWARE

1. Go to the GeneMapper Program Icon on the desktop and double click to load the program. Enter USER ID and PASSWORD. From the Project Manager Screen, go to the File menu and select ADD SAMPLES TO PROJECT. Select the appropriate data run files and click on ADD TO LIST. The run files are transferred to the right side of the screen. Click on ADD AND ANALYZE and give project the correct name including the run type and date run. Then click on OK.

2. The GeneMapper program automatically analyzes the run files using a sizing function based on the GS(400)HD-ROX size standard included in each sample.

3. If the program encounters a problem with the sizing standard table, it will flag the sample with a 'Low Quality' flag (red ball) in the sizing quality (SQ) column. Samples with the 'Low Quality' flag are placed at the top of the sample list. To correctly size the sample, go the ANALYSIS menu and click on SIZE MATCH EDITOR. In the Size Match Editor window, click on OVERIDE SQ and click on OK. The sample is then flagged with a blue X in the 'SQI' column.

4. Do the above to all samples with the 'Low Quality' flag. These samples must now be re-analyzed by GeneMapper. To re-analyze, go the ANALYSIS menu and click on ANALYZE. These samples are now ready for viewing.

5. To view individual run data, select a sample by highlighting it. Go the ANALYSIS menu and click on DISPLAY PLOTS. This opens the Sample Plots window.

6. For the T PCR, the PANES drop down should be on 2, then click on the "Separate Dyes" button. This displays all of the peaks detected for the 2 colors used in the assay. Master mix T1 is displayed in Black so turn off the colors Blue and Green. Master mix T2 is displayed in Green so turn off the colors Blue and Yellow. In both the T1 and T2 the ROX size standard is displayed in red. The display will show all of the peaks detected for the 2 colors used in the assay. Proceed to #9.

7. For the Size Ladder control (SL) reactions, use only 2 panes and turn off the green and yellow color graphs on the display by clicking on the color buttons.

8. The Size Ladder control (SL) reaction produces 6 peaks of the following sizes in normal human DNA samples with sufficient quantity and quality of DNA: 89bp, 96bp, 200bp, 300bp, 400bp and 600bp. Using the 400 ROX standard, the 600bp size, if present, is seen as 588bp because it is out of the dynamic range of the standard curve.

9. Each master mix (T1 and T2) has two size ranges (in bp) where product peaks are considered to be significant. For master mix T1, the valid size ranges are 55 to 85bp and 155-185bp. For master mix T2, the valid size ranges are 200 to 235bp and 235-270bp. The T2 multiplex master mix generates the products as <u>two distinct size ranges of products</u> and they should not be viewed as a continuum within the 200-270 range. A clonal sample will frequently generate a clonal

product in each of these size ranges. When products are generated in both of these size ranges the difference in size of these peaks is approximately 32 base pairs.

10. Analyze the NTC and Negative controls first. The NTC should give no major peaks in the size ranges of interest for any master mix. The Negative Control should show uneven, spiky but gaussian-like distributions in the valid size ranges.

11. The Positive control should show the control peak sizes listed by the manufacturer. These sizes are valid up to +/- 5bp.

12. If the controls are correct, then analyze each patient sample.

13. To save the analysis for review on another computer or to archive, it is necessary to save an image of the graphs in the sample plot window. The same procedure is used for the T PCR and the Size Ladder control reaction.

14. In the Sample Plot window, select the correct number of panes and split the dyes. Click on the keys Control 'G' to display only the selected sample peaks. In each electrophoretogram of T PCR, select any peaks that are of interest in the valid size range for each reaction. To be considered of interest, peaks found in the valid size range of a master mix reaction (T1 and T2) must be at least 3X the median peak height of the gaussian distribution of the range. Select no peaks if none are found in the valid size ranges of interest.

15. For the Size Ladder control reaction, only the expected size blue peaks are selected. Select no peaks if none are present where expected.

16. Click on F11 to open Capture Express software window. Select CAPTURE RECTANGULAR AREA to begin capture. Left click on mouse to begin area selection. Left click again to end capture. Select SAVE AS JPEG and then click on SAVE. Name image file with correct name and save in appropriate folder. Click on DONE to end capture.

17. Save all images for the run by transferring them to a CD using the CD writer.

18. Create results log for run. Include all peaks of interest for each patient.

F. RESULTS

Expected control results

The NTC should give no significant, major peaks in the size ranges of interest for any master mix. The Negative Control should show gaussian-like distributions in the valid size ranges. The Positive control should show the control peak sizes listed by the manufacturer. These sizes are valid up to +/- 5bp.

Reportable Range

Samples that contain a clonal population (Positive) in the T1 reaction, should show a distinct peak or peaks in the valid size ranges of 55 to 85bp and 155-185bp. Samples

that contain a clonal population (Positive) in the T2 reaction, should show a distinct peak or peaks in the valid size ranges of 200 to 235bp and 235-270bp. Samples containing polyclonal populations (Negative) should appear as gaussian-like distributions in the valid size ranges for each T multiplex PCR reaction.

Normal Values
Normal samples should contain polyclonal lymphocytic populations and should appear as gaussian-like distributions in the valid size ranges for each T multiplex PCR reaction. This is considered a Negative result.

Critical Values
There are no critical values associated with Multiplex PCR T Cell Gamma Gene Rearrangement Detection testing.

Analytic Interferences
Analytic interferences in PCR testing occur frequently. Certain fixative solutions yield DNA samples that are not compatible with PCR analysis, specifically B-5 fixative. Samples that do not amplify the adequate number of peaks in the Size Ladder control reaction may contain interfering substances that do not allow the proper functioning of the Taq polymerase enzyme. If the Size Ladder control reaction does not show the appropriate product larger than the upper limit of the valid size range for a reaction, the sample result is considered invalid and an answer of No Result is given (see below).

Final results are interpreted by the pathologist as follows:
Negative for T cell clonal peaks, positive for a T cell clonal peaks, or no result when the size Ladder control reaction does not show the appropriate size peaks.

G. LIMITATIONS

This Multiplex PCR method detects approximately 90% of T Cell gene rearrangements. Failure to detect some rearrangements might be attributable to insufficient homology between the T gamma V-region primer sequences and the T gamma V-region gene segment found in the rearranged DNA in question. The assay cannot reliably detect less than 1 positive cell per 100 normal cells.

ANNEXURE XII (A)

PROFORMA

Name: Age: Sex: S.No.

Address: Occupation:

Presenting Complaint:

Pruritus Yes/No

Skin lesions Yes/No

Others Yes/No

Duration of symptoms (in months)

Pruritus

Skin lesions

Others

Site of involvement- Face/trunk/upper limb/lower limb/genitalia/scalp/palms/soles

Systemic symptoms: Yes/No

	No	Yes	Duration
Fever			
Weight loss			
Cough			
Pedal edema			
Night sweats			
Others			

Any H/O drug intake- systemic/topical/native medications

Any immunosuppressants:

Last dose:

Examination

Type of skin lesions: Papules/macules/patch/Erythroderma/
Purpuric/tumors/nodules/bullae/ulcer

Size of involvement: Face/upper limb/lower limb/trunk/palms/
Soles/genitalia/scalp

Extent of involvement:

Sites	Yes	Total No	Sites of involvement
Face			
Upper limb			
Lower limb			
Trunk			
Genitalia			
Mucosa			
GS: Temperature	Pallor		

Systemic examination	Yes	No	Size
Lymph node			
Cervical			
Axillary			
Inguinal			
Abdomen			
Liver			
Spleen			
Ascites			

Investigations

1. Haemoglobin
2. TC
3. DC
4. Platelet count
5. ESR
6. Sezary cells
7. Urine

   M/E

   Albumin

   CXR
8. Bilirubin

SGOT

SGPT

ALK POS

LDH

9. Serum Creatinine
10. Serum Immune electroporesis
11. HbsAg
12. VDRL
13. HIV
14. HCV
15. Biopsy

a. Liver

b. Lymph node

c. Bone marrow

d. Skin

i) Epidermotropsim

ii) Density of infiltrate

iii) Cellular atypia

iv) Reticular fibroplasia of papillary dermis

v) Intraepidermal atypical lymphocytes

vi) Lymphocytic infiltrate without inflammatory features

vii) No. of elastic cells

viii) No. of mitoses MM

ix) Cellular density (No. of mononuclear cells/10mm)

x) Presence or absence of eosinophilia

xi) Follicular mucinosis

e) Immunophenotype

f) TCR gamma gene rearrangement – Monoclonal/Polyclonal/ Not done

Ultrasound-

Imaging (CT scan, MRI, PET scan)-

ANNEXURE XII (B)

KEY TO MASTER CHART

Variable	variable name	Code
idno	Unique ID	
name	Name of the patient	
age	Age of the patient	
agdia	Age at diagnosis	
sex	Sex	[Male=1, Female=2]
Hnum	Hospital number	
State	State	
occ	Occupation	
pcomp	Presenting complaint	
pru	Pruritus	[0=absent, 1=present]
sles	Skin lesions	[0=absent, 1=present]
oth	Others	[0=absent, 1=present]
dur	duration(in months)	
prud	Pruritus	[0-420 months]
slesd	Skin lesions	[0-420 months]
othd	Others	[0-420 months]
ssym	Systemic symptoms	[0=absent, 1=present]
fev	Fever	[0=absent, 1=present]
wlos	Weight loss	[0=absent, 1=present]
cou	Cough	[0=absent, 1=present]
pedem	Pedaledema	[0=absent, 1=present]
nsw	Night sweat	[0=absent, 1=present]
othss	Others	[0=absent, 1=present]
ptre	Past treatment	[0=no, 1=yes]
imms	Immunosuppressants	[0=no, 1=yes]
tsles	Type of skin lesions	
pap	Papules	[0=absent, 1=present]
hyoma	Macules	[0=absent, 1=present]
pat	Patch	[0=absent, 1=present]
pla	Plaques	[0=absent, 1=present]
pur	Purpura	[0=absent, 1=present]
poi	Poikiloderma	[0=absent, 1=present]
nod	Nodules	[0=absent, 1=present]
tum	Tumors	[0=absent, 1=present]
bul	Bullae	[0=absent, 1=present]
ulc	Ulcer	[0=absent, 1=present]
ery	Erythroderma	[0=absent, 1=present]
soi	site of involvement	
fac	Head and neck	[0=absent, 1=present]
tru	Trunk	[0=absent, 1=present]

```
ulim        Upper limbs [0=absent, 1=present]
llim        Lower limbs [0=absent, 1=present]
gen         Genitalia   [0=absent, 1=present]
pal         Palms       [0=absent, 1=present]
sol         Soles       [0=absent, 1=present]
muc         Mucosa      [0=absent, 1=present]
eoi         Extent of involvement [0=not applicable, 1=1-10%, 2=11-30%, 3=31-
50%, 4=51-80%, 5=81-100%]
plor        Pallor      [0=absent, 1=present]
lnod        Lymph node  [0=absent, 1=present]
cer         Cervical lymph node [0=absent, 1=present]
axi         Axillary lymph node [0=absent, 1=present]
ing         Inguinal lymph node [0=absent, 1=present]
liv         Liver enlargement   [0=absent, 1=present]
spl         Spleen enlargement  [0=absent, 1=present]
asc         Ascites             [0=absent, 1=present]
inves       Investigations
hb          Haemoglobin
wbc         Total WBC
neu         Neutrophils
lym         Lymphocytes
mon         Monocytes
eos         Eosinophils
bas         Basophils
mye         Myelocytes
memy        Metamyelocytes
atyl        Atypical lymphocytes [0-absent, 1-present]
plts        Platelets
esr         ESR
urin        Urine   [0=abnormal, 1=normal]
alb         S.albumin
bil         S.Bilirubin
sgot        Aspartate transaminases
sgpt        Alanine transaminases
alkp        Alkaline phosphatase
ldh         Lactate dehydrogenase
creat       Serum creatinine
hepb        Hepatitis B   [0=negative, 1=positive]
hiv         HIV      [0=non reactive, 1=reactive]
hepc        Hepatitis C    [0=negative, 1=positive]
vdr         VDRL     [0=non reactive, 1=reactive]
ana         ANA      [0=negative, 1=positive]
ebvp        EBV PCR  [0=negative, 1=positive]
htlv        HTLV-1 antibody  [0=negative, 1=positive]
sbio        Skin biopsy
```

epi	Epidermotropism	[0=absent, 1=present]
pmic	Pautrier microabscess	[0=absent, 1=present]
lymt	Lymphocytes tagging at DEJ	[0=absent, 1 present]
papd	Thickening of papillary dermis	[0=absent, 1=present]
alym	Atypical lymphoid infiltrate	[0=absent, 1=present]
lct	Large cell transformation	[0=absent, 1=present]
infmm	Inflammatory infiltrates	[0=absent, 1=mild, 2=moderate, 3=dense]
lich	Lichenoid band like infiltrates	[0=absent, 1=present]
sder	Superficial dermal	[0=absent, 1=present]
mder	Mid dermal	[0=absent, 1=present]
dder	Deep reticular dermal	[0=absent, 1=present]
scut	Subcutis	[0=absent, 1=present]
lob	Lobular	[0=absent, 1=present]
sep	Septal	[0=absent, 1=present]
pvas	Perivascular	[0=absent, 1=present]
ints	Interstitial	[0=absent, 1=present]
pfol	Perifollicular	[0=absent, 1=present]
padn	Periadnexal	[0=absent, 1=present]
pneu	Perineural	[0=absent, 1=present]
adir	Adipocyte rimming of lymphocytes	[0=absent, 1=present]
minf	Mixed inflammation	[0=absent, 1=present]
mit	Mitoses	[0=absent, 1=present]
muci	mucin deposits	[0=absent, 1=present]
haep	Haemophagocytosis	[0=absent, 1=present]
fatn	Fat necrosis	[0=absent, 1=present]
fmuc	Follicular mucinosis	[0=absent, 1=present]
angin	Angioinvasion	[0=absent, 1=present]
ihc	Immunohistochemistry	
cd3	CD3	[0=negative, 1=positive]
cd20	CD20	[0=negative, 1=positive]
cd30	CD30	[0=negative, 1=positive]
mib	MIB1 index	[0=1-10%, 1=11-30%, 2=31-50%, 3=51-80%, 4= >80%]
cd4	CD4	[0=negative, 1=positive]
cd8	CD8	[0=negative, 1=positive]
cd4c8	CD4 CD8 ratio	[0-not known, 1-CD4>CD8, 2-CD8>CD4, 3-equivocal]
cd5	CD5	[0=negative, 1=positive]
cd7	CD7	[0=negative, 1=positive]
cd45	CD45RO	[0=negative, 1=positive]
grb	Granzyme B	[0=negative, 1=positive]
cd56	CD56	[0=negative, 1=positive]
cd43	CD43	[0=negative, 1=positive]
cd79a	CD79a	[0=negative, 1=positive]
tia	TIA 1	[0=negative, 1=positive]
ebv	EBV LMP 1	[0=negative, 1=positive]
cd10	CD10	[0=negative, 1=positive]

bc2 Bcl-2 [0=negative, 1=positive]
cd23 CD23 [0=negative, 1=positive]
alk1 ALK [0=negative, 1=positive]
cd138 CD138 [0=negative, 1=positive]
tcrg TCR gene rearrangement [0=not applicable, 1=monoclonal, 2=polyclonal]
xra X ray [0=no extracutaneous involvement, 1=extracutaneous involvement, 9=not done]
usg Ultrasound [0=no extracutaneous involvement, 1=extracutaneous involvement, 9=not done]
cts CT scan [0=no extracutaneous involvement, 1=extracutaneous involvement, 9=not done]
mri MRI scan [0=no extracutaneous involvement, 1=extracutaneous involvement, 9=not done]
pet PET scan [0=no extracutaneous involvement, 1=extracutaneous involvement, 9=not done]
bmar Bone marrow biopsy [0=no evidence of lymphoma, 1=evidence of lymphoma present, 9=not done]
lyno Lymph node biopsy [0=no evidence of lymphoma, 1=evidence of lymphoma present, 9=not done]
orgb Extracutaneous Organ biopsy [0=no evidence of lymphoma, 1=evidence of lymphoma present, 9=not done]
ext Extracutaneous involvement [0=absent, 1=present]
aged Age at diagnosis [0-99 years]
durd Duration before first diagnosis [0-400 months]
msd Months since diagnosis [0-400 months]
trtr Treatment received [0-not known, 1-Chemotherapy, 2-PUVA, 3-Topical steroids, 4-Others]
stgd Stage of description [0=Prior to therapy, 1=recurrence or relapse, 2-disease persistent, 3-Improved/cured]
cat Category of lymphoma [0-NA, 1-primary CTCL, 2-secondary CTCL, 3-primary CBCL, 4-secondary CBCL, 5-precursor neoplasm]
diag Diagnosis [1-MF, 2-CD30 LPD, 3-SPTCL, 4-NKTL, 5-PTCLNOS, 6-MZBCL, 7-FCL, 8-DLBCLL, 9-DLBCLO, 10-ILBCL, 11-Blastic NKL]
mfdia Criteria of Mycosis fungoides [0-Other dermatoses, 1-MF cannot be excluded, 2-possibility of MF, 3-consistent with Mycosis fungoides]
mf Mycosis fungoides subtype [0-NA, 1-Classical MF, 2-PR, 3-Folliculotropic MF, 4-GSS, 5-Adult TCL, 6-Sezary syndrome]
cd30lp CD30 LPD [0-NA, 1-LyP, 2-ALCL]
ptcl PTCLNOS [0-NA, 1-Aggress epiderm CD8 TCL, 2-cut alpha/delta TCL, 3-small/medium pleomorphic TCL]
mtstg T staging MF [0-NA, 1-T1a, 2-T1b, 3-T2a,4-T2b, 5-T3, 6-T4]
mfnst N staging MF [0-N0, 1-N1, 2-N2, 3-N3, 4-Nx]
mfmst M staging MF [0-M0, 1-M1, 9-NA]
mfbst B staging MF [0-B0, 1-B1, 2-B2, 9-NA]

```
othstg      Staging other than MF   [0-NA, 1-done]
tstg        T staging   [0-T0, 1-T1a, 2-T1b, 3-T2a, 4-T2b, 5-T2c, 6-T3a, 7-
T3b]
nstg        N staging   [0-N0, 1-N1, 2-N2, 3-N3, 9=Nx]
mstg        M staging   [0-M0, 1-M1, 9=status not known]
mfstg       Final staging  [0-NA, 1-IA, 2-IB, 3-II, 4-IIB, 5-III, 6-IIIA, 7-
IIIB, 8-IVA1, 9-IVA2, 10-IVB]
```

ANNEXURE XII (C)

	age	agdia	sex	state	pru	sles	oth	prud	slesd	othd	ssym
1	63	58	1	Bihar	1	1	0	1	180		0
2	55	54	2	Tamilnadu	0	1	0		24		0
3	29	29	2	Tamilnadu	0	1	0		6		0
4	62	60	2	Jharkhand	1	1	0	6	6		0
5	22	22	2	Tamilnadu	0	1	1		8	0.3	1
6	14	14	1	Arunachal Pradesh	1	1	1	12	12	12	1
7	70	70	1	West Bengal	1	1	0	84	120		0
8	55	52	1	Jharkhand	1	1	0	120	120		0
9	44	43	2	Kerala	1	1	0	360	360		0
10	59	58	2	Bangladesh	0	1	1	0	2	1	0
11	43	43	1	Jharkhand	0	1	0		84		0
12	32	30	2	Mizoram	1	1	0	180	180		0
13	27	27	2	Tamilnadu	1	1	0	18	18		0
14	44	44	1	Tamilnadu	0	1	0		24		0
15	22	22	1	West Bengal	1	1	0	6	6		0
16	2	2	2	Chhattisgarh	1	1	0	2	12		0
17	41	41	1	Tamilnadu	0	1	0		180		0
18	53	53	1	Andhra Pradesh	0	1	0		60		0
19	70	67	1	Tamilnadu	1	1	0	72	72		0
20	6	5	1	West Bengal	1	1	0	36	36		0
21	56	54	2	Chhattisgarh	1	1	0	36	60		1
22	33	33	2	Tamilnadu	0	1	1		1	2	1
23	44	44	1	West Bengal	1	1	0	18	18		1
24	27	27	1	Tamilnadu	0	1	0		36		0
25	33	33	2	Karnataka	0	1	1		84	36	1
26	49	49	1	Chhattisgarh	0	1	1		0.5	3	1
27	19	19	2	Andhra Pradesh	0	1	0		6		0
28	14	14	2	Tamilnadu	1	1	0	120	120		0
29	30	30	1	Kerala	0	1	0		60		0
30	46	45	1	Kerala	0	1	1		0.3	0.5	1
31	40	40	1	West Bengal	0	1	1		24	0.5	1
32	26	26	1	Assam	0	1	0		180		0
33	74	74	1	West Bengal	1	1	0	96	96		0
34	41	40	1	West Bengal	0	1	1		18	12	0
35	62	62	1	Andhra Pradesh	0	1	1		48	4	0
36	42	42	1	Tamilnadu	0	1	1		36	24	0
37	59	59	1	Andhra Pradesh	1	1	0	30	30		0
38	12	12	1	Tamilnadu	0	1	0		6		0
39	50	49	2	Jharkhand	1	1	1	24	24	12	1
40	17	17	2	Karnataka	0	1	1		6	6	1
41	64	59	2	Kerala	1	1	1	180	180	154	0

42	77	77	1	Tamilnadu	0	1	0		6		0
43	23	19	2	Kerala	0	1	1		1	0.8	1
44	44	39	2	West Bengal	1	1	0	1	60		0
45	14	14	1	West Bengal	1	1	0	12	12		0
46	28	28	2	Andhra Pradesh	1	1	0	12	24		0
47	32	32	1	Tamilnadu	0	1	0		36		0
48	56	55	2	West Bengal	1	1	1	12	12	2	1
49	62	58	2	Kerala	0	1	0		72		0
50	70	65	1	Jharkhand	0	1	1		60	6	0
51	22	21	2	Tamilnadu	0	1	0		108		0
52	32	32	1	Maharashtra	1	1	0	180	180		1
53	52	52	1	Tamilnadu	0	1	0		5		0
54	43	41	2	Chhattisgarh	1	1	0		420		0

fev	wlos	cou	pedem	nsw	othss	ptre	imms	pap	hyoma	pat	pla	pur	poi
						0	0	0	1	0	0	0	0
						1	0			1			1
						1	0	1					
						0	0	0	0	0	0	0	0
1	0	0	0	0	0	1	1				1		
1	1	1	0	0	1	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	1	1	0	0
						1	0	0	0	1	0	0	0
						1	1	0	0	1	1	0	
						1	1				1		
						1	0	1	0				
						1	0			1			1
						1	0	1	1	1	1		1
						0	0	1	0	0	0	0	0
						1	0	1	0	0	0	0	0
						0	0	1	0	0	1	0	0
						1	0		1	1			
						0	0	0		1	0	0	0
						1	1	1		1			
						0			1				
			1			1	1	1	0	1	1	0	0
	1				1	0							
1	1	0	0	0	0	1	1			1	1		1
						1	0	1					
1	1	0	1	0	1	1	1						
1	1	0	1	0	1	0	0					1	
						1	0	0	0	0	0	0	0
						0		1		1	0	0	0
						1	0			1			

0	1	0	0	0	1	1	1						
1	0	1	0	0	1	1	1	0	0	0	1	0	
						1	0	1	0				1
						0	0			1			
						1	0						
						1	1			1	1		
						1	1	1	1				
						1	0			1	1		1
						0	0				1		
0	1	0	0	0	0	1	1	0	0	0	1	0	0
1	0	0	0	0	0	1	1	1	0	0	1	0	0
						1	1	0	0	1	1		1
						1	0				1		
1	0	0	0	0	0	1	1	0	0	0	0	0	0
						1	1	1	1	0	0	0	0
						1	0	0	0	1	0	0	0
						0	0	1	0	1	0	0	0
						0	0	0	0	1	0	0	0
1			1		0	1					1		
						1	0		1	1			
								0	0	0	1	0	0
						1	0	0	1	1	0	0	0
1	0	0	0	0	1	1	1	0	0	0	1	0	0
						1	0	0	0	0	0	0	0
						1	1	1	0	0	1	0	

nod	tum	bul	ulc	ery	fac	tru	ulim	llim	gen	pal	sol	muc	eoi
0	0	0	0	0	0	1	1	1	0	0	0	0	1
						1	1	1					2
							1	1					1
1	0	0	0	0	0	1	1	0	0	0	0	0	1
1						1		1					1
0	0	0	0	0	1	0	1	1	0	0	0	0	1
0	1	0	0	0	1	1	1	1	0	0	1	0	4
0	0	0	0	0	1	1	1	1					2
1			1		1	1	1	1		1	1	0	4
1	1		1	0	1	1	0	0	0	0	0	0	1
						1	1	1					1
						1	1	1	0	0	0	0	2
						1	1	1					2
0	0	0	0	0	0	0	1	0	0	0	0	0	1
0	0	0	0	0	0	1	0	0	0	0	0	0	1
0	0	0	0	0		1	1	1					1
						1	1	1					3

0	0	0	0	0	0	1	1	1					2
				1	1	1	1	1		1	0	0	5
					1	1	1	1	0	0	0	0	2
1	1				1	1	1	1		1	1	0	5
1						1		1					1
			1		1	1	1	1					4
						1	1	1					1
1					1	1	1	1	0	0	0	0	2
						1							1
1	0	0	0		1	1	1	1					1
0	0	0	0	0	1		1	1	0	0	0	0	3
					1	1	1	1					2
1					0	1	0	0	0	0	0	0	1
1	0	0	1		1	1	1	1					4
				1	1	1	1	1					5
						1	1	1					2
1	1		1			1	1						1
	1		1		1	1	1	1					3
					0	0	0	1					1
					1	1	1	1					4
								1					1
0	0	0	0	0		1	1	1		1	1		3
1	0	0	1	0			1	1					1
					1	1	1	1				0	3
1					1	1	1	1					3
1	0	0	0	0	0	0	0	1	0	0	0	0	1
0	0	0	0	0	0	1	0	0	0	0	0	0	1
0	0	0	0	0	1	1	1	1					4
0	0	0	0	0	0	1	1	1	0	0	0	0	2
0	0	0	0	0		1	1	1	0	0	0	0	4
1	1					1	1	1				0	2
						1		1					2
0	0	0	0	0	0	0	1	0	0	0	0	0	1
0	0	0	0	0	0	1	1	1	0	0	0	0	1
0		1	1		1	1	1	1	0	0	0	0	4
1		0	0	0	1	0	0	0	0	0	0	0	1
1		0	0	0	1	1	1	1		1	1	0	3

plor	lnod	cer	axi	ing	liv	spl	asc	hb	wbc
0	0				0	0	0	13	4400
0	0				0	0	0	13.5	9900
1	0				0	0	0	11.8	14500
0	1	1	1		0	0	0	7.8	5000
0	1	0	1	1	1	0	0	12.5	4100

0	1	1	0	1	1	1	0	11.2	5400
0	0				0	0	0	14.2	7300
0	0				0	0	0	15.3	6500
0	1	0	1	0	0	0	0	14.8	9400
0	1	1	0	0	0	0	0	10.3	15700
0	0				0	0	0	13.6	9400
0	0				0	0	0	16.3	5200
	0				0	0	0	12.3	9400
0	0				0	0	0	13.8	7800
0	0				0	0	0	16.1	6000
0	0				0	0	0	13	11800
0	0				0	0	0	13.7	9600
0	0				0	0	0		
0	1	0	1	1	0	0	0	13.4	8100
0	0				0	0	0	12.1	13300
0	1	1	1	1	0	0	0	13	19600
1	1	1	1	1	1	0	1	8.7	7400
0	1	0	1	1	0	0	0	10.1	10700
0	0				0	0	0	14.6	6800
0	0				0	0	0	9.1	2000
1	0				1	0	1	9.8	13700
0	1	0	1	0	0	0	0	11.8	6300
0	1	0	0	1	0	0	0	13.5	6200
0	0				0	0	0	15.3	11600
1	0				1	1	0	14.2	7600
0	1	1	1	1	0	0	0	11.5	9900
0	1	0	1	1	1	1	0	11.9	3100
0	0				0	0	0	11.6	7200
0	1	0	1	0	1	1	0	10.5	5500
0	0				0	0	0	11.1	7000
0	0				0	0	0	14.5	7000
0	0				0	0	0	14.2	6400
0	0				0	0	0	11.7	10400
0	0				0	0	0	11.9	5600
0	0				0	0	0	14.4	6010
0	0				0	0	0	10.5	6200
0	0				0	0	0	11	14100
0	0				0	0	0	13.7	5100
0	0				0	0	0	11.2	8600
0	0				0	0	0	14.3	7100
0	0				0	0	0	13.1	10500
0	0				0	0	0	13.5	10600
1	1	1	0	1	1	1		9.6	20800
0	0				0	0	0	11.9	10800

0	0				0	0	0	12.3	9800
0	0				0	0	0	11.6	5800
0	1	1	1	1	0	0	0	15.1	9700
0	0				0	0	0	14.7	9000
0	0				0	0	0	10.4	13800

neu	lym	mon	eos	bas	mye	memy	atyl	plts	esr	scell	urin	alb
70	18	8	4					103000				4.1
65	26	7	2					317000			0	4.9
60	31	9					0	271000			1	4.4
45	24	9	1					104000	69	0	1	3.8
67	24	7						225000	22	0	0	2.8
53	28		5				0	215000	17	0	1	2.5
51	19	12	18					165000	4	0		5
61	29	8	1	1			0	223000	1	0		4.7
71	17	4	8				0	179000				4.2
18	63	9	10					315000				4.4
67	23	6	4								1	
59	32	5	2	1			0	210000	1		1	4.5
66	25	6	2				0	276000	47			
61	29	5	4				0		25			
45	46	7	2				0					
28	62	7	3				0	348000				
76	14	9	1				0	218000	22	0	1	4.1
65	1	4					1	159000				3.9
55	29	8	8				0			0		4.9
34	58	6	2				1	243000	24	1	1	4.2
79	12	7	1	1				247000			1	2.9
66	11	11	12					320000	62	0	1	3.6
60	26	9	4	1				232000				4.2
70	19	11						296000	19	0	0	3.2
92	6	2						90000		0	1	2.1
48	37	13	2				0	185000	26	0	1	4.4
35	48	8	8	1				371000			1	4.7
52	35	8	4	1				352000		0		
62	30	7	1					145000			1	3.9
65	11	11	5					437000	60	0	1	2.7
45	36	0	8	1				55000		0	1	4.3
67	21	8	3					243000		0		4.4
79	6	14	1					148000				2.6
30	62	7		1			0	247000		0	1	3.4
68	22	8	2				0	260000			1	4.5
53	30	11	5	1			0	223000	14		1	4.9

64	29	6	1					308000				
63	22	9	5	1			0	172000	70			4.4
55	32	9	4				0					4.3
67	17	9	7				0	210000			1	4.1
39	17	20	6	1	17		0	69000	61	0	1	4.8
67	30	3					0	193000				
53	36	9	2				0	283000				4.5
56	26	10	7	1			0	232000				4.3
72	21	5	1	1			0	274000				4.4
63	17	9	10				0				1	4.4
22	69	5	2				1	117000				4.6
52	36	8	3				0	220000	31			4.6
50	33	7	10				0	129000			1	4.3
66	25	7	1				0		48			5
84	8	7	1				0	198000	5	0	1	3.7
42	50	6	2				0	281000	8			
43	38	8	11	0			0	258000				4

bil	sgot	sgpt	alkp	ldh	creat	imel	hepb	hiv	hepc	vdr	ana	ebvp	htlv
0.6	13	9	94	322	0.94								
0.4	15	13	50	408	0.72		0	0	0				
0.2	19	14	50		0.67		0	0	0		0		
0.4	27	12	80	425	0.99		0	0	0		1		
0.4	34	21	16	672	0.9		0	0	0		1		
0.8	182	162	1578	1413	0.61		0	0	0		0	1	
0.6	49	54	76	610	1.15								
0.4	23	24	64	311	1								
0.7	14	27	57	543	0.96								
0.4	39	40	143		0.55		0	0	0				0
				391	1.07					0			
0.4	31	14	30	452			0	0	0				
				586									
				527									
				316									
				754									
0.4	20	27	62	556	1.14		0	0	0		0		
0.5	31	19	62	1124	1.25								
	36	32	364		0.42		0	0	0				
0.2	24	12	68	1062	0.71		0	0	0				1
0.6	56	10	71	5850	1.11		0	1	0	0			
0.3	20	27	91	332	1.06		0	0	0		0		
0.5	17	11	77	457	1.25								

0.3	104	57	224	1144	0.31		0	0	0		0	0	
0.4	200	40	410	3310	1.42	0	0	0	0		0		
0.3	25	12	55	699	0.68		0	0	0		1		
	22	11	278	419	0.36								
				377									0
1.4	37	32	64	949	1.09		0	0	0				
0.7	15	24	61	700	0.82		0	0	0				0
0.5	17	21	51	332	0.81		0	0	0				
0.4	21	11	91	472	0.78								
0.6	30	23	128	567	0.84		0	0	0				
0.4	24	7	101	444	1.03		0	0	0				
0.4	20	17	65	388	1.19		0	0	0				0
0.3	22	20	67	334	1.02		0	0	0				0
							0	0	0				
0.4	37	31	102	585	0.79						0		
0.7	14	12	67	321	0.74								
0.3	25	28	103	386	0.93		0	0	0				0
0.3	24	13	85	733	1.19		0	0	0				
				1156			0	0	0		1		
0.5	55	53	134	399	0.8								
0.3	22	20	264										
0.5	20	15	60	407	1.05								0
0.4	25	22	104	441	1.36								
0.4	11	7	76	664	0.94		0	0	0				
0.5	27	28	47	595	1.2								
0.2	21	13	83	314	1.07		0	0	0		0		
0.5	19	10	90	524	0.74								0
1.3	35	53	55	1124	0.87		0	0	0		0		
				385									
0.8	14	6	59	470	1		0	0	0		0		0

epi	pmic	lymt	papd	alym	lct	infmm	lich	sder	mder	dder	scut	lob	sep	pvas	ints
1	0			1		1		1		0	0			1	
1	1	1		1		2		1			0			1	
0	0			1		2		1		1				1	1
0	0			1		2		0			1	1	0	0	
0				1		2					1	1	0		
0				1		3					0			1	1
1	1			1	1	3	1	1	1	1	0				
1		1		1		1		1			0			1	
1		1		1	0	2	1	1	1	1	1				
0				1		3					1		1		
0				1		2					0				

1	1	1		1		1		1			0				1
1		1		1		2		1						1	
0		1		1		2		1	1					1	
		1		1		2		1	1						
0	0	0		1		3		1		1				1	
1				1		1		1						1	
1		1	0	1		1		1						1	
				1		2	1	1						1	
1				1		1		1						1	
1	1			1	1	3	1	1	1						
				1		3			1	1					
1		1		1		3		1	1		1			1	
1				1		3		1	1					1	1
				1		2					1	1		1	
0				1		3		1		1	1	1	1	1	
				1		2				1	1	1			
1		1		1		2		1						1	
1	1	1		1		2		1							
				1					1	1	1				
		1		1		3									
1				1		3	1				1			1	
1		1		1		2	1	1						1	
				1		2					1				
1	1			1		3	1	1	1	1					
1		1		1		3					1				
		1		1		1		1						1	
0	1	1		1		1	1	1						1	
1	1	1		1		2	1	1							
0				1	0	2		1		1				1	1
1				1		1	1	1	1		0			1	1
				1		2		1	1						
				1		3				1	1				
				1		2		1						1	
1		1		1		2		1							
1		1		1		3		1		1					
1		1		1	0	2		1	0	0	0	0	0	1	
1				1		3			1	1					1
1		1		1		1		1						1	
				1		2					1	1		1	
1		1	1	1		2		1						1	
				1		2		1						1	1
1				1		3					0				
1	1			1		3					0			1	

pfol	padn	pneu	adir	plsc	minf	mit	muci	haep	fatn	angc	angin	cent	cenb	immun
				1	1	1								
			1				0		1					
			1	1	1	1	1	1			0			
1				1	1									
						1								
											0			
				1	1									
	1			1	1									
	1					1								
				1	1									
					1	1								
				1										
				1										
						1								
						1								
						1								
	1	1		1	1		0							
										1				
			1				0							
			1		1		1							
			1						1					
					1									
				1	1									
1														
	1					1								
					1									
						1								
				1	1	1								
1	1													
				1	1									

			1	1		1								
				1										
						1					1			
	1	1		1										
						1								
1	1					1					0			
				1	1									

cd3	cd20	cd30	mib	cd4	cd8	cd4c8	cd5	cd7	cd45	grb	cd56
1				1	1			0			
1				1	0		0	0			
0	0	1	1								
1	0	0	2	1	1	1					0
1	0		2	1	1						0
1	0	1			1			0		1	1
1	0	1	3	1	1	1		0			
1				1	1	1		0			
1	0	1	2	1	0			1			
1	0	1	2	1	0			0			
0	0	1									
1				1	1	1		0			
1				1	1	1		0			
1		1	1	1	0			0			
1	0	1	1								
1	0	1		1	1	1		0			
1				1	0			0			
1				1	1	1		0			
1	0	1	1	1	0	1					
1				1	1	2		0			
1		0	3	1	0	1		0			
0	0										
1		0		1	1	1		0			
1	0	1	2	1	1	2	0	0		1	1
1	0	0	3	1	1	2				1	0
1	0		3	0	1	2				1	0
1			2	1	1	2				1	0
1		0	1	1	1	1		0			
1				1	1	1		0			
0	0	1	4								

1		0	3	1	1	1		0		0	0
1			0	1	0			0			0
1				1	1	1		0			
1	0	1	3	1							
1		0	1	1	1	1		0			
1	0	1	3	1	0			0			0
1				1	0			0			
1				1	1	3		0			
1	0			1	1	1		0			
1		1	2	1	0						
1			1	1	1	1		0			
1	0	0	1	1	0	1		0			
1			2	0	1					1	
1	0	1									
1				1	1	1		0			
1				1	1			0			
1				1	1	1		0			
1	0	1	0	1	0						
1				1	1	2		0			
1	1	0	0								
1				1	0			0			
1	0	0	3	1	0			1			0
1		0	4	1	1	1		0		0	0
1	0		2	1	0						

cd43	cd79a	tia	ebv	cd10	bc2	cd23	alk1	cd138	cd123
		1	1						
0							0		

							0		
			1					1	
		1							
		1							
		1							
		1							
1	0						0		
		0							
							0		
							0		
							0		
								0	
0		0	0						

tcrg	xra	usg	cts	mri	pet	bmar	lyno	orgb	ext	aged	durd	msd	trtr
2	0	9	9	9	9	9	9		0	51	84	53	2
1	0	0	9	9	9	9	9		0	53	7	19	2
	0		9	9	9	9	9		0	28	6		
	0	0	9	9	9	0	0		0	59	6	18	1
2	0	0	9	9	9	0			0	21	2	5	3
	1	1	1	9	9	0	0	1	1	13	12		

	0	0	9	9	9	9	9		0	60	120		
1	1	0	1	0	0	9	9	9	0	42	120	40	2
			1	9	9	0	9		0	18	300	16	4
	0	0	9	9	9	1	9		1	58	1	7	1
	0	9	9	9	9	9	9		0	38	60		
1	0	0	9	9	9	9	9		0	12	220	24	2
1	0	0	9	9	9	9	9		0	25	18		
	0	0	9	9	9	9	9		0	43	9		
	9	9	9	9	9	9	9		0	21	12		
	0	9	9	9	9	9	9		0	1	12		
2	0	0	0	9	9	9	9		0	26	180		
		9	9	9	9	9	9		0	48	60		
	0	9	9	9	9	0	9		0	65	24	45	1
	0	9	9	9	9	9	9		0	4	24	14	3
	0	9	9	9	1	1	0		1	50	60	13	1
	0	1	9	9	9	1			1	33	1		
1	0	0	9	9	9	1	9		0	42	12		
	0	0	9	9	9	9	9		0	10	204	6	2
	0	0	0	9	0	0			0	26	84		
	0		0	0	9	0		0	0	49	0.5		
	0	0	0	9	9	0	9		0	18	6		
2	0	0	9	9	9	9	9		0	5	96		
1	0	0	9	9	9	9	9		0	24	72		
	1	1	1	1	1	0	9	1	1	45	0.5	6	1
	0	0		0	9	0			0	40	5		
		0	9	9	9	0	1		1	20	72		
	0	0	9	9	9	9	9		0	66	96		
	0	0	9	0	9	0	9	9	0	40	12	1	
	0	0	9	9	9	0	9	9	0	58	48	10	1
1	0	0	9	9	9	0	9		0	39	36		
2			0	0	9	9	9		0	56	18	4	2
	0	9	9	9	9	9			0	12	6		
	0	0	9	9	9	0	9		0	47	24	10	2
	9	0	9	9	9	9	9		0	16	6		
	0	0	9	9	9	9	9		0	53	72	53	1
	0	0	9	9	9	0	9		0	76	6		
		0				0	9		0	19	8	43	1
	0	0	9	9	9	0	9		0	39	4	65	1
1	9	9	9	9	9	9	9		0	13	12		
2	9	9	9	9	9	0	0		0	26	24		
	0	0	9	9	9	9	9		0	18	180	6	
		1				1			1	54	12	12	4
1	0		0						0	51	72	64	2
	0	0	0			1	9		1	65	6	68	4

1	0	0	9	9	9	0	9		0	72	108	18	2
	1	0	1			0	1	9	1	17	180		
	0		9	9	9	0	0	0	0	52	3		
	0	0	0	9	9	1		0	0	6	400	48	4

stgd	cat	diag	mfdia	mf	cd30lp	lyp	mtstg	mfnst	mfmst	mfbst	othstg	tstg	nstg	mstg	mfstg
2	1	1	1				3	0	0	0					2
2	1	1	3	1			3	0	0	0					2
0	1	2			1						1	5	0	0	2
3	1	3									1	7	1	0	4
2	1	3										6	9	0	4
0	2	4									1	7	3	1	10
0	1	1	3	1			5	0	0	0					4
3	1	1		1			3	0	0	0					2
2	1	1	2	1			5	4	0	0					4
1	2	2			2						1	7	1	0	4
0	1	2			1	0						7	0	0	4
3	1	1	2	1			3	0	0	0					2
	1	1	1	1			4	0	0	0					2
0	1	2			1	2						1	0	0	1
0	1	2			1	1						4	0	0	2
0	1	2			1							7	0	0	4
0	1	1	1				3	0	0	0					2
	1	1	3	1			3	0	0	0					2
1	1	2			2							7	9	0	4
2	1	1	1				3	0	0	0					2
1	1	1	3	1			6	1	0	1					7
	4	12										6	9	1	
0	1	1	1	1			5	4	9	1					4
1	1	2			1	4						7	0	0	4
0	1	3										7	0	0	4
0	1	3										4	0	9	
0	1	3										7	0	0	4
	1	1	1				3	4	0	0					2
0	1	1	1	1			3	0	0	0					2
1	2	2			2						1	6	1	1	
0	1	5										7	0	0	
0	1	1	3	1			6	3	0	0					9
0	1	1	2	1			3	0	0	0					2
0	1	2			2						1	4	9	0	
1	1	1		1			5	0	0	0					4
0	1	2			1	2					1	3	0	0	4
3	1	1	1	1			4	0	0	0					2
	1	1	1				2	0	0	0					1

2	1	1	3	1			4	0	0	0					2
	1	2			1	0						6	0	0	4
1	1	1	3	1			4	0	0	0					2
0	1	5										7	0	0	4
1	1	3										6	0	0	2
1	1	2			2							3	0	0	2
0	1	1	1	1			3	0	0	0					2
0	1	1	3	1			3	0	0	0					2
0	1	1	2	1			3	0	0	0					2
2	2	5										7	9	0	
3	1	1	2	1			3	0	0	0					2
1	3	9										4	0	1	10
3	1	1	3	1			1	0	0	0					1
0	2	5									1	7	3	0	9
0	1	5										1	0	0	1
2	1	1	3	1			4	0	0	1					3